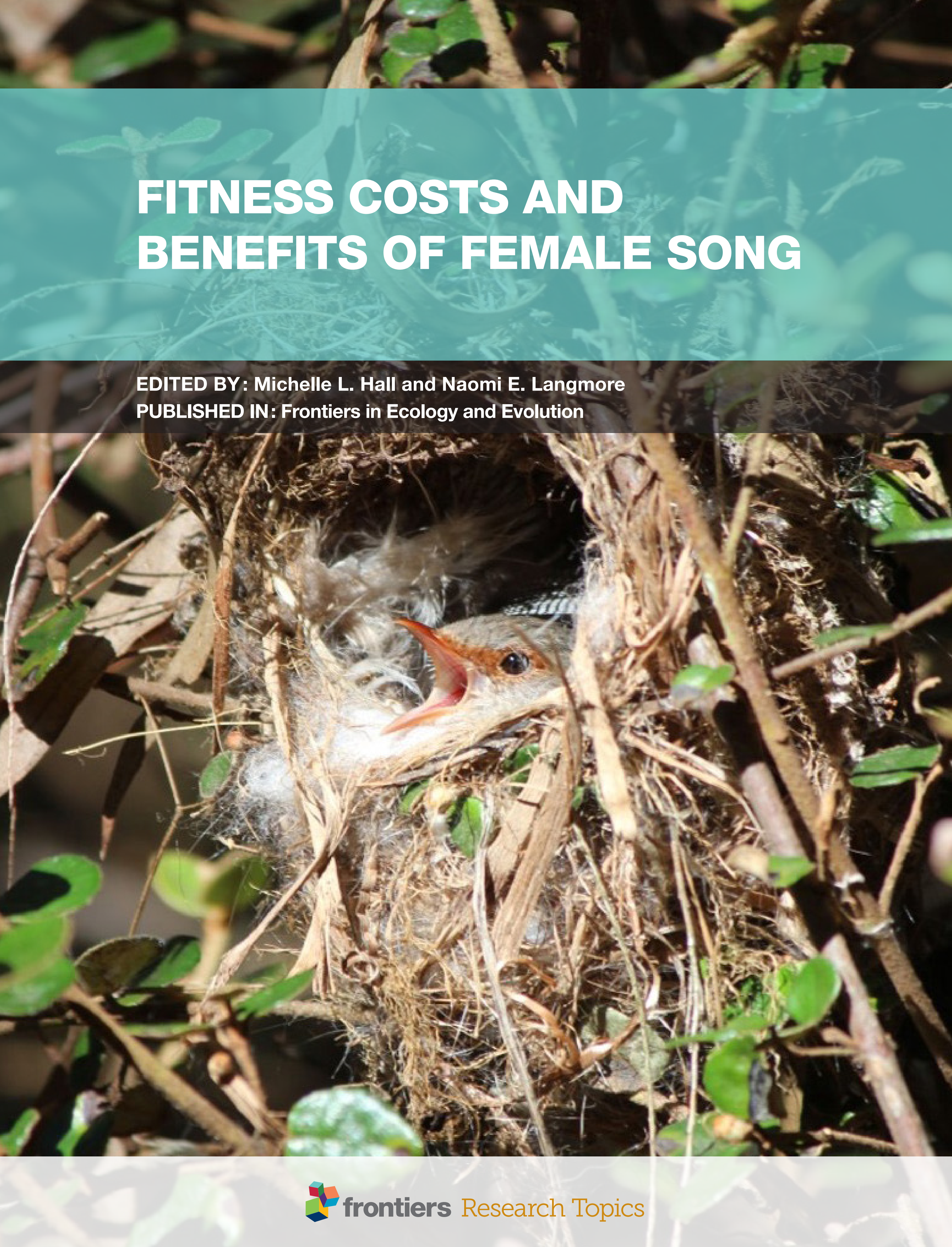

FITNESS COSTS AND BENEFITS OF FEMALE SONG

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FITNESS COSTS AND BENEFITS OF FEMALE SONG

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Females sing with males to form duets in Venezuelan troupials. Photo and audio: Karan Odom.

Cover photo: Female superb fairy-wren vocalises while on nest. Photo: Sonia Kleindorfer

Bird song, traditionally regarded as primarily a male trait, is in fact widespread among female songbirds and was probably present in the ancestor of modern songbirds (Odom et al 2014 Nature Communications). These recent findings challenge the view that sexual dimorphism in the expression and complexity of song is largely the outcome of sexual selection on males. It is now clear that understanding the evolution of bird song requires explaining variation within and among species in the expression and complexity of female song. To do this, we need a better understanding of the nature of variation in female song in different contexts and systems, as well as the fitness costs and benefits of variation in the expression and complexity of female song. This Research Topic draws together current research on female song with the goal of understanding the fitness costs and benefits of the diversity of female singing behaviour apparent among songbirds. It includes articles ranging from single-species studies investigating how female song varies with context and contrasts with male song, to comparative analyses exploring relationships between female song and ecological, social, and other factors, as well as opinion pieces.

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Photo: Lindley McKay. Audio: Michelle Hall

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Editorial: Fitness Costs and Benefits of Female Song

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Editorial on the Research Topic

Fitness Costs and Benefits of Female Song

The complexity and musicality of birdsong has fascinated naturalists for centuries. However, the historical research focus on temperate northern hemisphere birds has led to a widespread perception that song production is the domain of males alone. More recently, studies of birdsong have undergone a major paradigm shift, with the discovery that song in female birds is in fact widespread and ancestral, occurring in over 70% of surveyed species (Odom et al., 2014). This revolution in our understanding of song evolution has significant implications. First, it casts doubt on the view that song evolved primarily through sexual selection acting on males. Instead, the discovery that song occurred ancestrally in both sexes raises the possibility that song evolved initially for broader, social functions. Second, where sexual dimorphism in song production occurs, it is likely to reflect relatively recent loss of female song from an ancestor that had both male and female song (Odom et al., 2014). This shifts the focus of questions about sexual dimorphism in song production from "why do males sing?" to "why have females of some species lost song?." These issues are addressed in this Research Topic by Price, who argues that recent findings about the prevalence of female song do not conflict with previous research, but instead highlight some problematic assumptions about the evolution of sexual dimorphisms. These include misconceptions that current patterns of elaboration and diversity in each sex reflect past rates of change and that levels of sexual dimorphism necessarily reflect levels of sexual selection.

Given the changing focus in studies of birdsong evolution, a better understanding of how selection acts on song in females is sorely needed. Specifically, we need to understand the fitness costs and benefits of female song, yet there is an almost complete dearth of studies on this topic. The contributions presented in this special issue approach this topic from a diversity of angles, encompassing measures of the relationship between song and fitness, between song and other display traits and between male and female songs, as well as investigations of how females learn their songs.

FEMALE SONG AND FITNESS

Two papers in this Research Topic investigate the association between song production and reproductive success in wild birds. Brunton et al. found that in New Zealand bellbirds *Anthornis melanura* female song rates during both the incubation and chick-rearing phases strongly predicted the number of fledged chicks. Similarly, Cain et al. showed that female song rate in response to playback was positively related to nest success and the number of fledglings produced in superb fairy-wrens *Malurus cyaneus*. Both these studies provide evidence of a link between female song and fitness. However, further work is needed to identify the nature of this link. Do high song rates and more complex songs improve resource acquisition and increase reproductive success directly, or are

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they independently linked to another variable, such as female condition or age? A third paper sheds some light on this question. Keen et al. demonstrate that in superb starlings *Lamprotornis superbus* song diversity is correlated with age and breeding experience. In many bird species, nesting success improves with age and there is a concomitant preference for pairing with older mates (Kokko, 1997). This could, in turn, select for song elaboration as a signal of age and experience in both sexes (Langmore et al., 1996; Ballentine, 2009). These studies generate many fascinating hypotheses to explain the relationship between female song, age and breeding success. An important next step is to conduct experimental studies to elucidate the causal nature of the relationship.

HOW DO FEMALES LEARN THEIR SONGS?

Studying the ontogeny of female song in the wild is challenging because females are usually the dispersive sex in birds (Greenwood, 1980). However, this life-history difference between the sexes may influence the fitness costs and benefits of different song learning strategies if females experience greater changes in social environment associated with natal dispersal. Riebel highlights the importance of studying song learning processes for developing hypotheses for the function and evolution of female song. Knowing who females learn their songs from, and when they learn, are topics explored by three studies in this volume. Evans and Kleindorfer assessed element sharing rates in free-living superb fairy-wrens and found that females, like males, shared elements with both their male and female parents, suggesting that learning in this species does not involve sex-specific lineages. Dalziell and Wellbergen found that female superb lyrebirds *Menura novaehollandiae* imitate vocalizations of a wide range of other species, and use mimicry in a context-dependent way. Dowling et al. address the question of the timing of sensitive periods for vocal learning, and suggest that vocal learning in red-backed fairy-wrens *Malurus melanocephalus* may begin very early in life, since offspring songs are similar to maternal incubation calls. With many decades of laboratory studies and increasing numbers of field studies on vocal learning in males, we are just at the beginning of teasing apart song learning in females to discover similarities and differences with what we know about males.

Understanding the biological basis of song in male birds has been vital for investigating the relationship between song and fitness in males. The developmental stress hypothesis linked large song repertoires with large vocal areas in the brain associated with high resource availability during early development, so that repertoire size serves as an honest indicator of male quality (Buchanan et al., 2003). Whether such links between the biological basis of song and fitness exist in female birds is yet to be determined. In this issue, Lobato et al. address the biological basis of song ontogeny in female and male blue-capped cordon-bleus *Uraeginthus cyanocephalus* with a neuro-anatomical study. Their finding that brain sexual dimorphism is not always paralleled by song dimorphism highlights the importance of considering females as well as males for understanding the complexity of brain-behaviour dynamics.

FEMALE SONG AND OTHER DISPLAY TRAITS

Another promising avenue for elucidating the fitness benefits of female song is to examine song in the context of other display traits. For example, is female song associated with colourful plumage, or behavioural displays? Webb et al. demonstrate that across songbird species, female song occurs mainly in those species that exhibit elaborate plumage colouration in both sexes. This result suggests that the two signalling modes have reinforcing, overlapping functions and that they have evolved together in response to similar selective pressures. These findings contrast with those of two other studies in this volume. Mahr et al. tested the relationship between song and plumage characteristics in relation to female quality in superb fairy-wrens. They found no relationship between song variables and UV-colouration and proposed that the two traits evolved independently in that species. This may occur when different traits reveal different information about the signaller and are driven by different selective pressures (the "multiple messages" hypothesis). Similarly, Soma and Garamszegi tested the relationship between song, plumage and dance in Estrildid finches and found that the three traits were not correlated in either sex. However, expression of these traits was correlated between the sexes; species that have males with one or more exaggerated traits also have females that express the same traits. Like Mahr et al.'s results, this study suggests that multiple signals arise as a result of different selection pressures.

WHEN FEMALES AND MALES SING

The effect of communication within and between established breeding partnerships on fitness is often neglected in studies on birdsong. Research on female song sometimes ignores this complexity, either investigating female song function in the same theoretical framework typically used to investigate male song, ignoring the role of the partner, or studying female song in the context of duetting at the level of the pair rather than the individuals. As Logue and Krupp highlight, it is particularly important to distinguish individual- and pair-level perspectives in studies of duetting because if partners combine their vocalisations to form duets, there are multiple pathways by which female song might affect fitness.

Comparative studies identifying life-history and ecological correlates of female song and duetting across a wide range of species can also shed light on the fitness costs and benefits of female song. Previous work has shown that gains of migratory behaviour are typically associated with losses of female song and duetting (Logue and Hall, 2014). However, Najar and Benedict's comparative analysis show that, in a group where migration is the ancestral state, losses of migratory behaviour are not associated with gains of female song. They highlight the need for information about pair bond duration and territoriality throughout the annual cycle to better understand the evolution of female song. Tobias et al. also use phylogenetically controlled comparative analysis to assess evolutionary correlates of duetting and chorusing at a global scale. Including both songbirds and non-songbirds, their analysis shows that the likelihood of females vocalising together with males and other group members is most strongly associated with year-round territoriality, and not with latitude, migration, climate variables, or habitat openness.

Thus far, comparative analyses have investigated evolutionary correlates only of the presence or absence of female song. To better understand factors driving the elaboration or loss of acoustic ornaments, we need to know more about variation in female song rates and complexity within and across species. Odom et al. provide such valuable information in a within-species comparison of the structure and contexts of female and male song in the Venezuelan troupial *Icterus icterus*. Studies on female song often involve species with elaborate female song or duets, with less known about cases where female song is not as elaborate. In banded wrens *Thryophilus pleurostictus*, where females sing at much lower rates and with less complexity than males, Hall et al. find that female song seems to function primarily for communication between partners, rather than to repel rival females or to attract males. However, further work is needed to determine if and how female song used in this context affects fitness.

CONCLUSION

The diversity of contributions to this Research Topic highlight the breadth of research currently being conducted on female song. They also reveal how much further behind we are in understanding the functions, ontogeny, mechanisms, and evolution (Tinbergen, 1963) of birdsong when it comes to females, compared to males. We hope that this Research Topic stimulates further research to improve our understanding of the fitness costs and benefits of birdsong with more work on females to redress this imbalance.

AUTHOR CONTRIBUTIONS

MH conceived the topic idea, and MH and NL developed the topic, contributed to overseeing peer review of contributions, and wrote the editorial article.

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Rethinking our assumptions about the evolution of bird song and other sexually dimorphic signals

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Bird song is often cited as a classic example of a sexually-selected ornament, in part because historically it has been considered a primarily male trait. Recent evidence that females also sing in many songbird species and that sexual dimorphism in song is often the result of losses in females rather than gains in males therefore appears to challenge our understanding of the evolution of bird song through sexual selection. Here I propose that these new findings do not necessarily contradict previous research, but rather they disagree with some of our assumptions about the evolution of sexual dimorphisms in general and female song in particular. These include misconceptions that current patterns of elaboration and diversity in each sex reflect past rates of change and that levels of sexual dimorphism necessarily reflect levels of sexual selection. Using New World blackbirds (Icteridae) as an example, I critically evaluate these past assumptions in light of new phylogenetic evidence. Understanding the mechanisms underlying such sexually dimorphic traits requires a clear understanding of their evolutionary histories. Only then can we begin to ask the right questions.

Keywords: ancestral, female song, phylogeny, sexual dichromatism, sexual dimorphism, sexual selection

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Introduction

Male songbirds sing to attract mates and compete with rivals, and since Darwin these elaborate vocalizations have been widely acknowledged to have evolved through sexual selection (Darwin, 1859; Andersson, 1994). Research has long supported this premise by revealing aspects of male song production and complexity that are favored by females during mate choice and that benefit males during interactions with territorial neighbors (Marler and Slabbekoorn, 2004; Catchpole and Slater, 2008). Sexual dimorphism in song is generally assumed to be the outcome of sexual selection acting on males, whereas female singing has often been dismissed as anomalous (e.g., Beecher and Brenowitz, 2005; Byers and Kroodsma, 2009). Thus, alongside elaborate male plumage displays, male bird song has become a textbook example of a sexually-selected ornament.

Recent findings, however, appear to challenge this view. Female bird song is much more common than previously thought (Langmore, 1998; Riebel et al., 2005; Garamszegi et al., 2007; Price et al., 2009; Odom et al., 2014), especially among tropical species that defend year-round territories (Morton, 1996; Slater and Mann, 2004; Price, 2009). An extensive survey across songbirds has revealed the occurrence of female singing in over two thirds of surveyed species including 32 avian families (Odom et al., 2014). Earlier perceptions of bird song as a primarily male trait are now thought to reflect past geographical biases among researchers toward temperate passerine species, which are disproportionately well-studied and in which songs are produced almost exclusively by males (Morton, 1996; Riebel, 2003). Even among tropical species, female singing might be widely underreported because many species with female song also have sexually monomorphic plumage and year-round territorial defense by both sexes, so female singers could be frequently mistaken for males (Price et al., 2008). Indeed, considering the higher avian biodiversity in the tropics, the occurrence of female song may be the rule rather than the exception among songbirds.

Furthermore, phylogenetic studies have shown that sexual differences in singing behavior are often the product of historical losses in females rather than gains in males (Price et al., 2009; Odom et al., 2014). In the New World blackbird family (Icteridae), for instance, evolutionary reconstructions show that both sexes sang at similar rates in ancestral taxa and that female song has since been lost multiple times independently across the clade, resulting in a large proportion of today's species in which songs are produced primarily by males (Price, 2009; Price et al., 2009). Female song probably also existed in the ancestor of all modern songbirds, suggesting that the absence or presence of female singing among many current taxa is the outcome of either past losses in females or the evolutionary maintenance of this behavior in both sexes (Odom et al., 2014).

Altogether, these findings call into question the long-standing assumption that sexual dimorphism in song is the outcome of sexual selection acting on males. But does this suggest that previous researchers, including Darwin himself, have been wrong about the evolution of male bird song through sexual selection? Here I contend that these new findings are surprising not because they contradict previous results but because they disagree with some of the assumptions we tend to make regarding the evolution of sexual dimorphisms in general and female song in particular, assumptions that may not be accurate. These include: (1) viewing sexual dimorphism as a discrete rather than continuously variable attribute, and thus underestimating its prevalence across taxa; (2) conflating evolutionary rates of change with levels of selection; (3) conflating current levels of elaboration and divergence in each sex with past evolutionary rates; (4) assuming that levels of sexual dimorphism necessarily reflect levels of sexual selection; and (5) viewing decreases in dimorphism, in song or in other traits, as evolutionary gains in one sex rather than losses of sex-specific developmental mechanisms.

These potential misconceptions may apply to other sexually-selected signals that can occur in both sexes, such as conspicuous plumage colors. The New World blackbirds have served as a model clade for investigating a variety of such sexual dimorphisms, including the evolution of male-female differences in color (Irwin, 1994; Hofmann et al., 2008; Friedman et al., 2009; Price and Eaton, 2014), size (Webster, 1992), and song (Price, 2009; Price et al., 2009; Odom et al., 2015). Here I focus on this avian group in particular to examine how our new understanding of the evolutionary history of bird song compares with that of other sexually dimorphic traits, especially sexual dichromatism, and how these patterns line up with our past assumptions.

Female Singing Should Not Indicate a Lack of Sexual Dimorphism in Song

Although female song is often treated as a discrete binary character in comparisons among species, with some species categorized as having female song and others not (e.g., Garamszegi et al., 2007; Price, 2009; Odom et al., 2014), evidence suggests that female singing can vary continuously across taxa in both expression and complexity. For example, the New World blackbird clade includes species in which female song has never been recorded (*Sturnella neglecta*), others in which females rarely sing (*Icterus galbula*), species in which both sexes regularly produce similar songs (*Agelaius assimilis*, *Icterus pustulatus*), and even species in which pairs combine their songs into highly coordinated duets (*Dives dives*) (Price et al., 2009; Odom et al., 2015). Thus, like sexual size dimorphism (Webster, 1992), sexual differences in song production in this family appear to fall along a broad continuum.

The treatment of female song as a discrete rather than continuous trait parallels approaches used in some previous comparative studies of sexual dichromatism in birds, in which species have been categorized as either dichromatic or monochromatic (e.g., Burns, 1998; Friedman et al., 2009; Soler and Moreno, 2012) despite observations that differences between the sexes can vary continuously. In fact, analyses of plumage reflectance data reveal that many differences in color are imperceptible to humans and that truly sexually monochromatic bird species are surprisingly rare (Eaton, 2005, 2007). The New World blackbirds again provide an example. Although many species in this clade are considered sexually monochromatic (Jaramillo and Burke, 1999), nearly all exhibit at least some sexual color differences based on thresholds of avian color discrimination, and across taxa the sexes vary from nearly indistinguishable to strikingly different along a continuous range (Hofmann et al., 2008; Price and Eaton, 2014).

Likewise, although female song occurs widely across songbird species (Odom et al., 2014), true sexual monomorphism in singing and in associated neuroanatomy might be relatively uncommon (MacDougall-Shackleton and Ball, 1999; Garamszegi et al., 2005). Even if female song is more widespread than we currently realize, it seems likely that most males and females differ in this trait in some way, such as in production rates or acoustic structure. These differences deserve further attention. Thus, like the occurrence of colorful pigments in female plumage, the occurrence of song in females should not necessarily imply a lack of sexual dimorphism in this behavior. Song may occur in both sexes but nevertheless may reflect selection pressures and patterns of evolutionary change that are sex-specific.

Rates of Evolutionary Change Do Not Necessarily Indicate Levels of Selection

Several studies have shown that male-female differences in singing behavior are often the product of historical changes in females rather than males (Price, 2009; Price et al., 2009; Odom et al., 2014). This again parallels studies of sexual dichromatism in birds, which show that striking male-female color differences are often the outcome of past losses of conspicuous plumage colors in females rather than gains of these colors in males (Irwin, 1994; Burns, 1998; Wiens, 2001; Hofmann et al., 2008; Price and Eaton, 2014). Dramatic female changes have also occurred when dichromatism has decreased, with females evolving rapidly to look like males and males changing comparatively little over time (Johnson et al., 2013; Price and Eaton, 2014). Based on these patterns, one might assume that selection on females has been stronger than selection on males.

Yet, a lack of recent change in a trait should not necessarily indicate that it has not been favored by selection. Although selection is generally viewed as a driving force of evolutionary change, many adaptive phenotypic characters are under strong stabilizing selection and presumably have remained unchanged for long periods of evolutionary history (Price and Lanyon, 2002; McGlothlin et al., 2010). As an example, numerous studies show that the songs and conspicuous red epaulets of male red-winged blackbirds (*Agelaius phoeniceus*) are important sexually-selected signals that clearly influence male success (Searcy and Yasukawa, 1995). Nevertheless, phylogenetic analyses including other species in the *Agelaius* genus suggest that the buzzy songs, brightly colored epaulets, and glossy black plumage of males are ancestral traits that have changed relatively little since the common ancestor of this clade (Barker et al., 2008; Price et al., 2009; Price and Eaton, 2014). In contrast, female vocalizations and colors differ substantially among species (Jaramillo and Burke, 1999), some appearing very similar to males (*A. assimilis*, *A. xanthomus*, *A. humeralis*) and others strikingly different (*A. phoeniceus*, *A. tricolor*), reflecting significant past changes. Presumably, these rapid female changes have had no direct bearing on how male traits have been influenced by sexual selection.

Current Levels of Diversity Do Not Necessarily Reflect Past Rates of Change

Phylogenetic reconstructions of male and female plumage color evolution show that the sexes exhibit very different modes of change, with male colors diverging steadily over time and female colors changing periodically and relatively rapidly either away from or toward male patterns, resulting in respective increases or decreases in dichromatism (Johnson et al., 2013; Price and Eaton, 2014). These sex-specific evolutionary modes might reflect different mechanisms of selection, with male colors continuously changing through sexual selection (Prum, 1997; Price and Whalen, 2009) and female colors showing a more punctuated pattern of change through natural or social selection, perhaps based on levels of intra-sexual competition or the need for female crypsis near the nest (Martin and Badyaev, 1996; Amundsen, 2000; Soler and Moreno, 2012; Tobias et al., 2012).

Patterns of song variation reveal similar evolutionary differences between the sexes, with male song characteristics showing steady rates of divergence (Price and Lanyon, 2002, 2004) and females producing either male-like songs or very different vocalizations (Price et al., 2009; Odom et al., 2015). Indeed, among many New World blackbird taxa, the evolution of song and color dimorphisms have occurred largely in parallel. All species with frequent female song also exhibit relatively low levels of dichromatism (Jaramillo and Burke, 1999; Price and Eaton, 2014). On the other hand, not all species that are dimorphic in song are likewise dimorphic in plumage, suggesting that the selective factors favoring male-female differences in these traits are not necessarily the same (also see Soma and Garamszegi, 2015).

A surprising result of these sex-specific evolutionary modes is that male traits can appear significantly more elaborate and divergent among species than do female traits, despite the fact that females have changed more frequently and dramatically in the evolutionary past (Price and Eaton, 2014). While the males of different taxa have accumulated novel plumage features over time, for example, females have converged repeatedly on similarly cryptic color patterns, resulting in relatively dull female plumages that appear as if they have not changed much at all (Martin and Badyaev, 1996; Hofmann et al., 2008; Price and Eaton, 2014). In a similar way, male song features have evolved along multiple complex trajectories (Price and Lanyon, 2002, 2004), while losses of female song have produced outcomes that are largely the same across taxa (Price, 2009). Clearly, current levels of trait complexity and diversity are not good indicators of past rates of change.

Sexual Dimorphism Is Not Due to Sexual Selection Alone

Altogether, recent phylogenetic comparative studies provide strong evidence against the widely-held assumption that sexual dimorphism is driven by sexual selection alone. Even when levels of dimorphism correspond closely with social mating systems across taxa (Webster, 1992; Irwin, 1994; Dunn et al., 2001; Price and Eaton, 2014), male-female differences in any particular species are the product of different selective pressures acting on each sex and not simply strong selection on males (Badyaev and Hill, 2003; Cox and Calsbeek, 2009; Dunn et al., 2015). Indeed, dimorphisms in both song and plumage colors have been shown to be associated with an assortment of life history traits besides mating system, including breeding latitude, seasonal migration, nesting behavior, and territoriality (Martin and Badyaev, 1996; Friedman et al., 2009; Price, 2009; Soler and Moreno, 2012; Johnson et al., 2013; Odom et al., 2015). Nevertheless, the perception that levels of sexual dimorphism reflect levels of sexual selection remains pervasive, even among some researchers (e.g., Seddon et al., 2013; Bloch, 2015).

Levels of sexual dimorphism in a trait may have little direct relationship with the functions of that trait in attracting or competing for mates. Plumage features in a variety of passerine species are known to strongly influence male reproductive success but nonetheless differ little between the sexes (e.g., Hill et al., 1999; Doucet et al., 2005). Levels of dimorphism may also show little relationship with past rates of evolutionary change. In the oropendolas and caciques, for example, a clade within the New World blackbird family, plumage colors have evolved more rapidly in polygynous than monogamous taxa, presumably due to sexual selection, yet none of these species are notably dichromatic (Price and Whalen, 2009).

Is Song Dimorphism the Result of Evolutionary Gains or Losses?

All autosomal genes spend half their evolutionary histories in males and half in females, on average and assuming equal sex ratios. Many genes have influences on the phenotype regardless of sex, whereas others have influences that are sex-specific and result in phenotypic attributes that are sexually dimorphic (Coyne et al., 2008). The evolution of such sex-specific genetic influences on the phenotype involves special selective mechanisms in which expression is favored in one sex and concurrently opposed in the other (Kimball and Ligon, 1999; Badyaev and Hill, 2003; Ketterson et al., 2005; Cox and Calsbeek, 2009). Secondary losses of sexual dimorphism in turn involve losses of these sex-specific molecular pathways, through changes in one or both sexes to match the other (Wiens, 2001).

By this logic, increases in dimorphism through losses of female singing behavior, as has occurred in many songbird lineages (Garamszegi et al., 2007; Price et al., 2009; Odom et al., 2014), involve the evolution of novel, sex-specific molecular pathways in females that secondarily influence the development of male-like traits. This is reflected in neurodevelopmental patterns, in which males and females initially develop equivalent neural song control systems which subsequently atrophy in the females of species that lack female song (Konishi and Akutagawa, 1985). Thus, from a developmental perspective, losses of female song and the neuroanatomical modifications associated with this change could be viewed as evolutionary gains. Contrariwise, decreases in song dimorphism through evolutionary gains of female song could be viewed as developmental losses, if the evolution of male-like singing in females involves the loss of female-specific neurodevelopmental pathways and behaviors.

We should expect such losses of dimorphism to be especially rapid given that much of the genetic and hormonal architecture for male-like song production is presumably already present in females (Lande, 1980; Kimball and Ligon, 1999; Ketterson et al., 2005). Increases in song dimorphism, in contrast, should occur more gradually since this involves the accumulation of novel traits in males or females, or both, through natural or sexual selection.

Evolutionary reconstructions of sexual dichromatism in both the New World blackbirds (Price and Eaton, 2014) and the distantly related Australian fairy wrens (Maluridae; Johnson et al., 2013) are largely consistent with these predictions. In both families, decreases in dichromatism have involved females rapidly gaining male-like characteristics rather than the reverse, whereas increases in dichromatism have tended to occur more gradually and involve changes in either sex. It would be interesting to investigate such patterns in the evolution of male and female song.

A variety of selective mechanisms have been offered to explain the evolution of elaborate songs in females, including mutual mate choice, intra-sexual competition, and other forms of social selection (Langmore, 1998, 2000; Tobias et al., 2012). Yet, few of these hypotheses address why female songs tend to closely resemble those of conspecific males. The ideas presented here do not disagree with these hypotheses, but rather build on them by suggesting that selection for female song may occur especially rapidly since it favors genes and molecular pathways that may already exist. Understanding the mechanisms of past changes can help us to ask the right questions in understanding current traits.

Conclusions

Recent studies are prompting us to reconsider long-held assumptions about the evolution of male and female song. Rather than being anomalous, female singing is widespread among songbirds and indeed was probably present during the early evolution of bird song (Odom et al., 2014). Past geographic biases and other factors appear to have misled us toward the opposite perspective, that male-only song is the norm and that evolutionary changes in song have occurred primarily in males. Given our new perspective, we face exciting new challenges to understand the selective mechanisms underlying the diversity and evolutionary history of female song (Riebel et al., 2005; Price, 2009; Odom et al., 2015). Indeed, in many well-studied bird species, we should probably now be asking why females do not sing rather than why males do.

But these new findings should not necessarily alter our view of bird song as a model for studies of sexual selection. As hopefully conveyed here, past rates of female change and current levels of dimorphism may have little relevance to how male traits have been influenced by sexual selection. In fact, if anything, these new insights into our previous misconceptions are instructive and only further serve to illustrate what an effective research model bird song has become.

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FEMALE SONG AND FITNESS

Female song rate and song complexity are correlated with breeding success in New Zealand bellbirds.
Photo: Aaron Harmer

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Female Song Rate and Structure Predict Reproductive Success in a Socially Monogamous Bird

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Bird song is commonly regarded as a male trait that has evolved through sexual selection. However, recent research has prompted a re-evaluation of this view by demonstrating that female song is an ancestral and phylogenetically widespread trait. Species with female song provide opportunities to study selective pressures and mechanisms specific to females within the wider context of social competition. We investigated the relationship between reproductive success and female song performance in the New Zealand bellbird (*Anthornis melanura*), a passerine resident year round in New Zealand temperate forests. We monitored breeding behavior and song over 3 years on Tiritiri Matangi Island. Female bellbirds contributed significantly more toward parental care than males (solely incubating young and provisioning chicks at more than twice the rate of males). Female song rate in the vicinity of the nest was higher than that of males during incubation and chick-rearing stages but similar during early-nesting and post-breeding stages. Using GLMs, we found that female song rates during both incubation and chick-rearing stages strongly predicted the number of fledged chicks. However, male song rate and male and female chick provisioning rates had no effect on fledging success. Two measures of female song complexity (number of syllable types and the number of transitions between different syllable types) were also good predictors of breeding success (GLM on PC scores). In contrast, song duration, the total number of syllables, and the number of "stutter" syllables per song were not correlated with fledging success. It is unclear why male song rate was not associated with reproductive success and we speculate that extra-pair paternity might play a role. While we have previously demonstrated that female bellbird song is important in intrasexual interactions, we clearly demonstrate here that female song predicts reproductive success. These results, with others, highlight the need for a change in how we view the significance of female secondary sexual traits; traits long underestimated due to a focus on male song.

Keywords: female song, parental investment, reproductive success, social selection, song rate, song structure, song performance, female-female competition

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INTRODUCTION

Darwin (1871) described bird song as a male trait that has evolved through sexual selection. Since then, research has focussed on temperate, northern hemisphere passerines where song has been seen as a male activity, functioning in territoriality, and mate attraction (Catchpole, 1982; Searcy and Andersson, 1986; Kroodsma and Byers, 1991). However, as the number of worldwide studies of songbirds has increased, the phenomenon of complex, territorial female song has been found to be more widespread than first thought (Arcese et al., 1988; Langmore, 1998; Riebel, 2003; Riebel et al., 2005; Brunton and Li, 2006; Garamszegi et al., 2007; Price et al., 2008, 2009; Geberzahn et al., 2009). Indeed, recent research has prompted a re-evaluation of the assumption that song is a male trait by demonstrating that female song is an ancestral and phylogenetically widespread trait in the oscines (Odom et al., 2014; Price, 2015). These findings raise the issue of why female song may have been lost in some species and the possibility of sex differences in not just the benefits of singing but also in the costs (Kleindorfer et al., 2016).

Male song has been extensively studied and there is evidence that measures of male song quality and performance are related to mating success (Searcy and Marler, 1981; Catchpole, 1987; Lampe and Espmark, 1994; Reid et al., 2004) and reproductive success (Catchpole, 1986; Reid et al., 2005; Nemeth et al., 2012; Taff et al., 2012; Woodgate et al., 2012). Researchers have also found links between male song performance and genetic benefits to females (Hasselquist et al., 1996). It has also been suggested that male song can act as a proxy for age (Botero et al., 2009; Kipper and Kiefer, 2010; Nemeth et al., 2012). However, not all studies of male song have found a relationship with reproductive success (Hiebert et al., 1989; Rivera-Gutierrez et al., 2010; Soma and Garamszegi, 2011), highlighting that plumage and other behavioral traits may play a more significant role in demonstrating male quality.

In contrast to male song, female song performance and structure is a long-neglected field (Langmore, 1998; Riebel, 2003; Riebel et al., 2005). Nonetheless, in some species, females are prolific singers (e.g., Cooney and Cockburn, 1995; Pavlova et al., 2005; Brunton and Li, 2006; Soma and Garamszegi, 2015). In several species where both sexes sing, the responses of territory holders may be sex specific, with same sex interactions being typical (Langmore and Davies, 1997; Langmore, 1998; Brunton et al., 2008b; Kleindorfer et al., 2013; Odom et al., 2015). In general however, the functions of female song are not well understood but have been suggested to parallel those associated with male song (Langmore, 1998), particularly in regard to social competition for resources other than a mate i.e., territorial defense (West-Eberhard, 1983; Illes, 2015). Sexual selection is most commonly defined as a process functioning via intrasexual competition for mates and mating opportunities (Clutton-Brock, 2007). Consequently, traits such as female song that may increase the competitive ability of individuals and be important in female-female competition are not included within this narrow definition of sexual selection. However, female-female competition is covered by the broader approach of social selection (West-Eberhard, 1979, 1983; Amundsen, 2000; LeBas, 2006; Kraaijeveld et al., 2007; Rosvall, 2011; Tobias et al., 2011). In species where females contribute more to rearing offspring than males, female-female competition is arguably highest during breeding activities and in dense populations. If characteristics of female song reflect competitive ability, then we predict an association between female song rate and reproductive success and a positive association between aspects of song performance and female fitness. To our knowledge, just one study has examined female song rates and fitness (Cain et al., 2015), and it found a positive relationship between defense responses to song playbacks during territory establishment and the probability of successful nesting. No study has yet examined how female song complexity and song rate during breeding relates to reproductive success.

Our study uses observational data on patterns of female and male song rates, female song complexity, and amount of parental care to examine the association between these variables and reproductive success (a proxy for fitness). We focused on female song in the vicinity of the nest and spontaneous song rates and song complexity in the New Zealand bellbird (here-after referred to as bellbird); an endemic honeyeater with well-developed female territorial song. For bellbirds, intrasexual aggression is an important component of social interaction for both sexes (Brunton and Li, 2006) and previous playback experiments show that females respond aggressively to female song playbacks that simulate territorial intrusions (Brunton et al., 2008a), particularly to playbacks of their female neighbors (Brunton et al., 2008b). Singing and counter-singing by females occurs frequently throughout the breeding season and females often sing upon leaving the nest after an incubation bout (Li, 2002). We used observations of song and parental investment, and structural analysis of song of individual females to test the hypothesis that female song rates and complex female songs are associated with reproductive success. We suggest that female song performance during the breeding season is crucial in competitive social interactions associated with the defense of resources (including male parental investment), which are essential for breeding activities. Therefore, we predicted a positive relationship between female song rate and/or song structure and reproductive success.

METHODS AND MATERIALS

Study Area and Species Background

Our study was conducted on Tiritiri Matangi Island (Tiri), a 220 ha island in the Hauraki Gulf, 28 km north of Auckland (Lat. 36°60′S, Long. 174.89°E). The bellbird population on Tiri is high-density, with over 10 birds/ha (Baillie and Brunton, 2011; Baillie et al., 2014), and has existed on the island for at least 100 years. Tiri is a wildlife sanctuary with open access to the public and currently has no introduced mammalian predators. The island has been extensively replanted over the last 20 years and a series of avian and reptilian species have been reintroduced for conservation reasons (Rimmer, 2004). Tiri is typical of most northern New Zealand forest ecosystems with year round fruiting and flowering of plant species providing ephemeral, scattered patches of abundant food resources (Gravatt, 1970). Consequently the social behavior and breeding chronology of the avian species and particularly honeyeater species is closely linked to the spatial and temporal patterns of plant breeding phenology (Craig and Douglas, 1986).

Bellbirds are sexually dimorphic in plumage and song (Heather and Robertson, 2000) and easily distinguished in the field (females are approximately 20% smaller and paler gray-green than males, and have a white cheek stripe). Relatively little is known about bellbird breeding biology (Kendrick, 1994; Brunton and Li, 2006; Massaro et al., 2008), although we do know that bellbirds are socially monogamous and that both sexes defend territories and/or resources throughout the year, but particularly during the breeding season (Craig and Douglas, 1986; Kendrick, 1994). The breeding cycle of bellbirds begins in late August and extends until January (Anderson and Craig, 2003). Clutch sizes range from 2 to 5 eggs and several clutches are laid each season. Nest-building activities (2–3 weeks) and incubation (2 weeks) are solely by females, whereas nest-based chick rearing is by both sexes and lasts until chicks fledge at 14–20 days of age. Chick provisioning by both parents can extend past fledging from the nest (Anderson and Craig, 2003). Although only the females incubate the eggs, males feed their mates during this period (Kendrick, 1994).

Bellbird Song

The singing modes of female and male bellbirds differ. Males frequently sing prolonged bouts during dawn chorus and engage in synchronized male chorusing at food sources (Li, 2002). At other times, males sing discrete songs and countersing with other males (Li, 2002). Females sing discrete songs with intervals of at least 3 s between songs (Li, 2002). Although female singing is rarely synchronized, counter-singing by nearby females occurs frequently and females often sing on leaving the nest after an incubation bout (e.g., Li, 2002). Although the number of song types produced by male and female bellbirds is similar (approximately 10 for the Tiri population), this variety is produced in different ways (Brunton and Li, 2006). Females produce song types that have the same basic structure but use different combinations and repetitions of syllables (e.g., ABC, AABC, ABCDE, AABCD, etc.). In contrast, each male song types are structurally distinct and may contain song type-specific syllables. Because of this difference between males and females in song type structure, and the low rate of singing by males in the vicinity of the nest, we were not able to obtain a large enough sample of male song and could not include measures of male song complexity. We based all syllable and song type definitions on previous bellbird studies (Li, 2002; Brunton and Li, 2006).

Data Collection

We collected the data used in this study as part of a larger study of bellbird behavioral ecology that has been running since 2007. The breeding season is during the austral summer, from October to late January. We monitored 26 nests (all first clutches of the season) by color banded males and females on Tiri over three breeding seasons 2010/2011, 2013/14, and 2014/2015 for a total of 216 h ($\bar{x} = 8.6 \pm 0.2$ h/nest; **Table 1**). We checked nests regularly (every 2–4 days) to determine clutch size, and hatching and fledging success. We captured adults using mist-nets or specially modified catching cages containing sugar-water feeders (each cage had multiple trap door entrances that we released manually when a target bird entered the cage). As part of our regular banding protocol we measured weight (g), wing length (mm), tarsus length (mm), tail length (mm), head-bill length (mm), body fat (0–3), and molt score (presence/absence), then we applied color-bands and collected a blood sample (all handling, banding, and blood sampling was performed under permits from the New Zealand Department of Conservation and the Massey University Animal Ethics Committee).

TABLE 1 | Summary of nest observations over the 3 years of the study.

Breeding stage		Year			Overall
		2010/2011	2013/14	2014/15	
EARLY-NESTING					
Hours	Total	30			30
	Mean ± SE	3.2 ± 0.1			
Nests	ID	1–5			
	n	5			5
INCUBATION					
Hours	Total	30	10	26	66
	Mean ± SE	4.2 ± 0.3	5 ± 0.7	4.1 ± 0.3	
Nests	ID	1–9	11, 14	19–23	
	n	9	2	5	16
HATCHING					
Hours	Total	40	44	32	116
	Mean ± SE	4.0 ± 0.3	5.8 ± 0.4	4.0 ± 0	
Nests	ID	1–10	11–18	19–26	
	n	10	8	8	26
POST-BREEDING					
Hours	Total	22			22
	Mean ± SE	5.5 ± 0.1			
Nests	ID	1–10			
	n	4			4

We compiled two independent datasets to examine female song and reproductive success. The first dataset involved direct observations of banded females and males in the vicinity of the nest to determine female and male song rates and behaviors associated with nesting activities (see below). Where possible, observations of song rates were also conducted 2 weeks prior to nesting and 2 weeks post-breeding. The second dataset used opportunistic audio recordings of color-banded females collected during breeding attempts and with known outcomes, to investigate the relationship between song structure and reproductive success.

Observations of Song Rate (Songs per Hour) and Nesting Behavior

Nests were observed continuously for durations of 1 h from clear vantage points at a distance of 5–10 m using binoculars. All activities within 15 m of the nest were generally visible. Incubation and chick-rearing stages were analyzed separately, and only nests with at least 3 h (max 8 h) of observation in a given stage were included (**Table 1**). Nests were not observed twice in the same day, observations started from approximately 8 a.m., and time of day was randomized. Observations for each nest spanned the entire nesting cycle but were not done systematically with regard to development stage. Behaviors recorded during 1 h observation periods for males and females included song bouts, and three parental investment behaviors: male and female chick provisioning, and male feeding the incubating female. All rates are reported per hour (averaged per nest per stage). Only nests that successfully hatched (but not necessarily fledged) at least one chick were included in the analysis of song rates and nesting behavior. Due to small sample sizes during early-nesting and post-breeding stages, overall comparisons of male and female song rates across breeding stages were compared using Wilcoxon signed-rank tests.

Female Song Recordings

We made song recordings of color-banded females using a Marantz PMD661 sound recorder and a Sennheiser ME66 K6 shotgun microphone (frequency response 50–22,500 Hz ± 2.5 dB). The microphone was hand-held using a Rycote shock-mount with a pistol grip. Songs were analyzed using spectrograms and waveforms generated in Raven Pro 1.5 (Cornell Laboratory of Ornithology, Ithaca, NY). Spectrograms were created using the Hann algorithm (Filter bandwidth 500 Hz, size 256 samples, and time gird overlap 50%). Five song measures (**Figure 1**) were taken from each of three songs per female and an average for each female was calculated. Three of the measures represented aspects of duration: song length (s ± 0.01), total number of syllables, and the number of "stutter" syllables ("stutter" syllables are short syllables that distinctively start and end female songs; Brunton and Li, 2006). The other two measures were proxies for song complexity: number of syllable types, and the number of transitions between syllable types.

Statistical Analysis

We investigated the effects of female and male song rate and parental investment on reproductive success (number of chicks fledged) using Poisson regression with a log-link function. Average hourly song rates per nest per stage were calculated and included in the models. Incubation and chick-rearing stages were analyzed separately, with female song rate, male song rate and the rate of male feeding the incubating female as predictors during the incubation stage, and female song rate, male song rate, female chick provisioning rate, and male chick provisioning rate as predictors during the chick-rearing stage. Males only feed females during the incubation stage. Analysis showed that during the incubation stage, female song rate, and male feeding rate were correlated (**Figure 2A**) and so a reduced model was fitted with male feeding rate removed. For the chick-rearing stage, there was a correlation between female song rate and male provisioning rate (**Figure 2B**), and so again a reduced model was fitted with male provisioning rate removed. There were no significant interactions in either analysis and so interactions were excluded from the models.

The effect of each of the song measures on nesting success (1) or failure (0) was examined using a Mann-Whitney U test. The relationship between female song structure and reproductive success (number of chicks fledged) was also investigated using Poisson regression with log-link function. However, the five song structure measures (song length, total number of syllables, number of syllable types, transitions between syllable types, and number of stutter syllables) were not independent and so a principle components analysis (PCA) was performed to reduce dimensionality of the data and generate independent predictor variables. Principle components one and two accounted for approximately 80% of the variation in the data, approximately evenly distributed between the two. Principle component loadings (**Table 2**) indicated that PC1 was composed mainly of

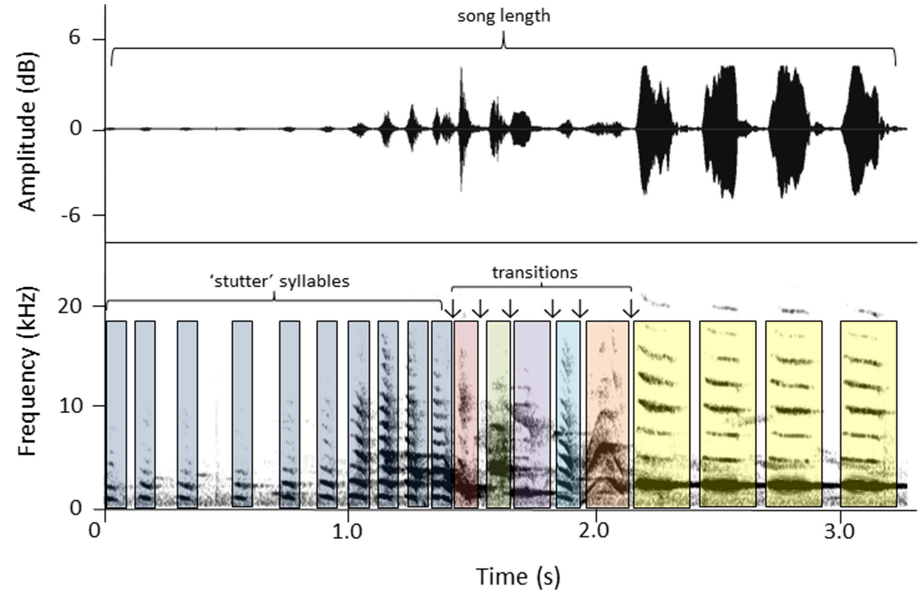

FIGURE 1 | An example of a female song (waveform above, spectrogram below) and the song parameters calculated for each song: song length (s), total number of syllables, syllable types (colors represent different type), number of transitions between syllable types (↓), and number of stutter syllables. Measures for this song example are: # syllables = 19, song length = 3.19 s, # syllable types = 7, # stutter syllables = 10, # syllable transitions = 6.

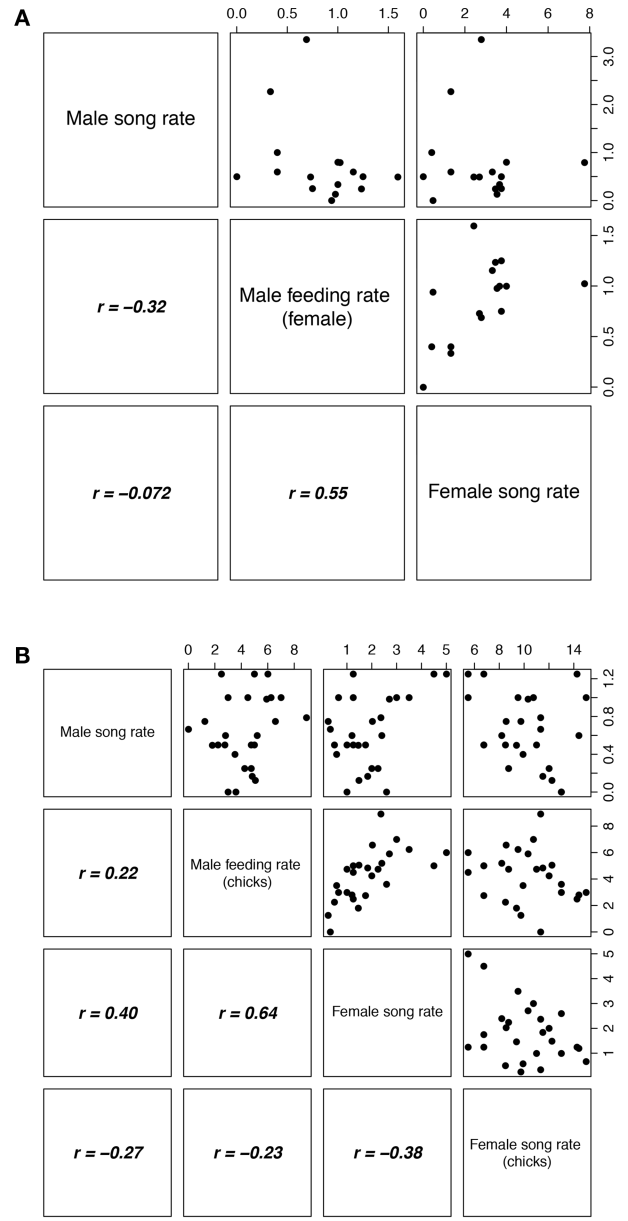

FIGURE 2 | Correlation matrix for behavioral parameters measured during (A) incubation and (B) chick-rearing stages. The four behavioral measures are on the diagonal, the upper triangle shows scatter plots, and the lower triangle shows the respective Pearson's *r*-values. All values were measured as frequency per hour. Dataset based on means per nest (incubation, 16 nests; chick-rearing, 26 nests).

total number of syllables and stutters per song, while PC2 was composed of the number of syllables types and the number of transitions per song. The principle component scores for PC1 and PC2 were then used as predictors in the Poisson regression. All analyzes were performed using the programming environment R (R Core Team, 2015). Where reported, means are presented with standard errors (± SE) and a significance level of $\alpha = 0.05$ was used for all statistical tests.

TABLE 2 | The loadings of the five song measures on PC1 and PC2 from the PCA analysis.

	PC1	PC2
Song length	−0.474	−0.239
Number of syllables	−0.625	0.192
Number of syllable types	−0.191	−0.646
Number of stutter syllables	−0.583	0.297
Number of syllable transitions	−0.089	−0.633

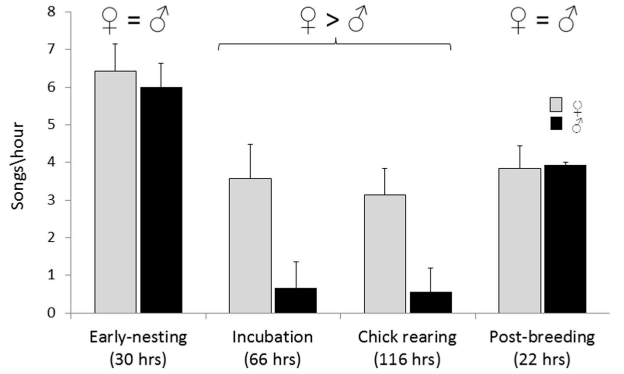

FIGURE 3 | Spontaneous song rate (songs per hour) by males and females on nesting territories (< 15 m from the nest site) based on 234 h of observations of 26 nests. Wilcoxon signed-rank tests were used to compare song rates of males and female within each breeding stage. For early-nesting and post-breeding stages the differences were non-significant, and for incubation and chick-rearing stages female song rates were significantly greater than male song rates, $W = 0$, $P < 0.05$.

RESULTS

Female Song Rates and Reproductive Success

Song rates (per hour) on nesting territories varied with breeding stage and were not significantly different between males and females during early nesting and immediately post-breeding (Wilcoxon signed-rank test, $W = 0$; **Figure 3**). In contrast, during incubation and chick rearing stages female song rate was significantly higher than male song rate (*Wilcoxon signed-rank test*, $W = 7$ and 8, respectively, $P < 0.01$; **Figure 3**).

Observations of 16 nests during incubation (66 h) and 26 nests (the 16 incubation nests plus 10 additional nests) during chick-rearing (116 h) showed that female bellbirds contribute significantly more parental care than males (solely incubating young and provisioning chicks at more than twice the rate of males; *paired t-test:* $t = 8.22$, $df = 26$, $P < 0.0001$; **Figure 4**). Female song rate during both incubation and chick-rearing was a significant predictor of the number of chicks fledged (**Table 3**; **Figure 5**), but male song rate was not (**Table 3**). Female chick provisioning was not a significant effect in our model (**Table 3**).

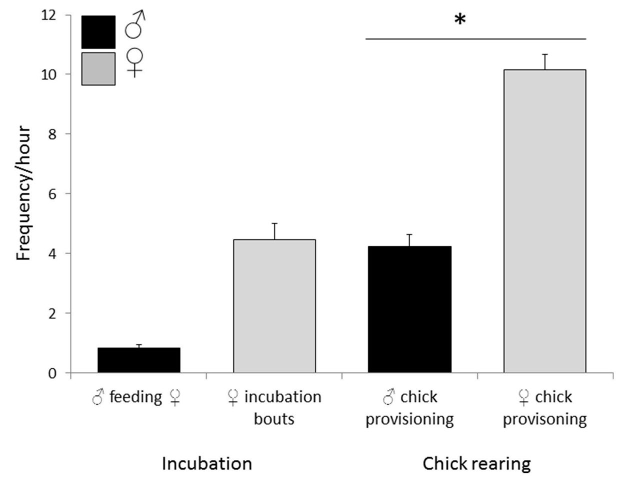

FIGURE 4 | Relative parental investment (PI) by male and female bellbirds. During incubation PI included the male feeding incubating female and female incubation bouts (16 nests, 66 h). During chick-rearing PI included male and female provisioning chicks (26 nests, 116 h). Female chick provisioning rate was significantly greater than male chick-provisioning frequency (*paired t-test:* $t = 8.22$, $df = 25$, $P < 0.0001$, indicated by *). Only nests that hatched at least one chick were included in the analysis.

TABLE 3 | Results of generalized linear models relating fledging success to song and provisioning rates, and song structure.

	Effects	Estimate ± SE	*P*
Fledging success (incubation)	Female song rate	0.23 ± 0.1	0.016
$\chi^2 = 5.79$, $n = 16$, $P = 0.055$	Male song rate	−0.19 ± 0.3	0.559
Fledging success (chick rearing)	Female song rate	0.46 ± 0.2	0.006
$\chi^2 = 9.57$, $n = 26$, $P = 0.023$	Male song rate	−0.1 ± 0.5	0.830
	Female provisioning rate	0.84 ± 0.1	0.269
Fledging success (song structure)	PC1	−0.12 ± 0.2	0.458
$\chi^2 = 17.33$, $n = 19$, $P < 0.001$	PC2	−0.86 ± 0.3	0.001

Chi-squared values calculated by comparing final model with null model.

Female Song Structure and Reproductive Success

Fifty-seven high quality recordings (low noise to signal ratio) of single songs by 19 females (three songs per female), where nesting outcome was known, were included in the analysis. We first examined breeding success as a binary response (failed vs. one or more fledglings) for the five song structure measures, and found that only song complexity (number of syllable types and number of transitions between syllable types) were significantly different (*Mann-Whitney U-tests*: $U = 4$, $P < 0.05$, and tests $U = 18$, $P < 0.05$, respectively; **Figure 6**). We then included fledgling number (0–3) and applied our GLM model using PCA scores from the five song measures and found that PC2 was a predictor of the number of chicks fledged (**Table 3**) and PC1 was not (**Table 3**). The number of different syllable types and the number of transitions between different syllable types were both associated with PC2 (**Table 2**; **Figure 7**). Hence, the more syllable types and syllable transitions in a female's song, the greater number of chick fledged (**Figure 8**). The duration of the song, the total number of syllables, and the number of introductory stutter syllables did not predict nesting success.

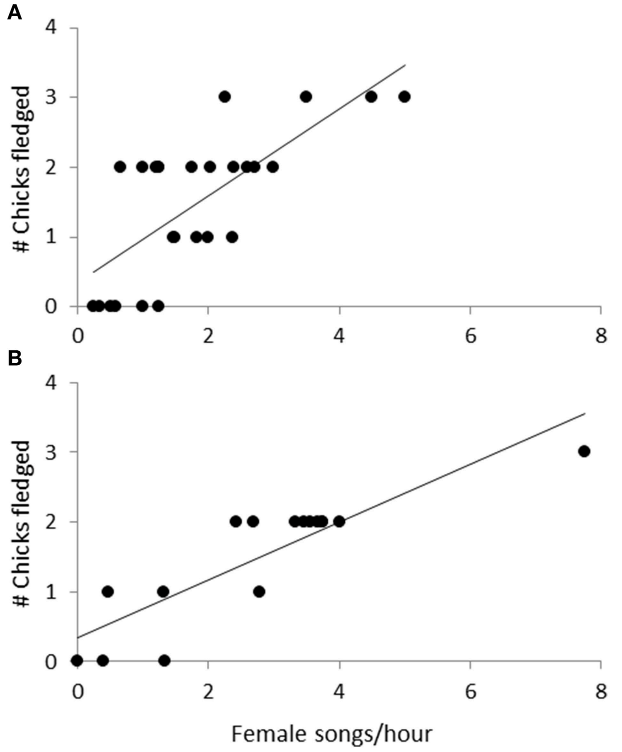

FIGURE 5 | The relationship between the number of chicks fledged and female song rate during (A) incubation ($n = 16$, 66 observation hours), and (B) chick-rearing ($n = 26$, 116 observation hours). The line is from a regression.

DISCUSSION

We examined the relationship between reproductive success and measures of song structure in females and found that spontaneous song rate during breeding positively correlated with reproductive success and that measures of female song complexity (number of different syllable types and number of transitions between different syllable types) predicted greater breeding success in bellbirds. This contrasts somewhat with other studies where female territorial defense behavior in response to a simulated female intruder (but not spontaneous song), predicts reproductive success (Cain et al., 2015), and where higher rates of female song at the nest increases predation risk (Kleindorfer et al., 2016).

It is known that female song in bellbirds has an important role in female competition (Brunton et al., 2008b) and here we found that higher female song rate during both incubation and chick-rearing were positively correlated with male provisioning rate (**Figure 2**). In northern cardinals (*Cardinalis cardinalis*),

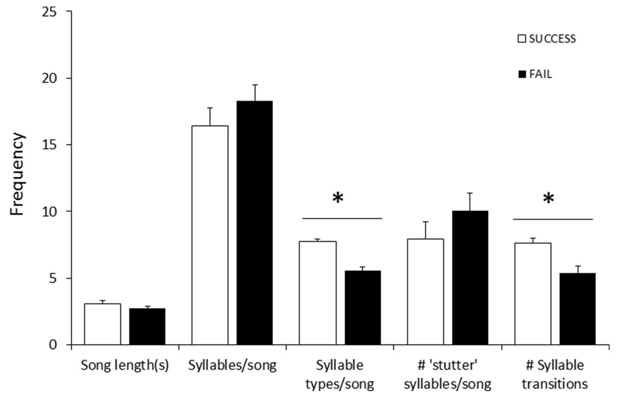

FIGURE 6 | Differences in female song measures (song length, number of syllables, number of syllable types, number of transitions between syllable types, and number of "stutter" syllables; averaged using three songs per female) between nests that successfully fledged chicks (□, $n = 10$) and nests where eggs hatched but no chicks fledged (■, $n = 9$). Data included nests from 2013/14 ($n = 9$) and 2014/15 ($n = 10$), all by different females. There was no difference between years in the proportion of successful or failed nests (2013: 5 successful, 4 failed; 2014: 4 successful, 5 failed). Significant differences (Mann-Whitney U-test) are indicated (*).

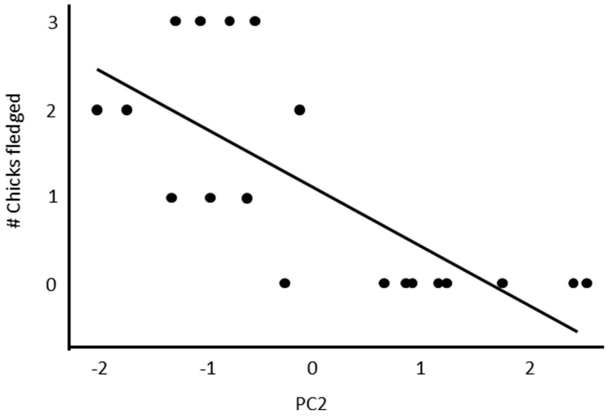

FIGURE 7 | The relationship between the PC2 scores of a PCA analysis and reproductive success. Table 2 indicates the loadings of each of the five song parameters on PC2.

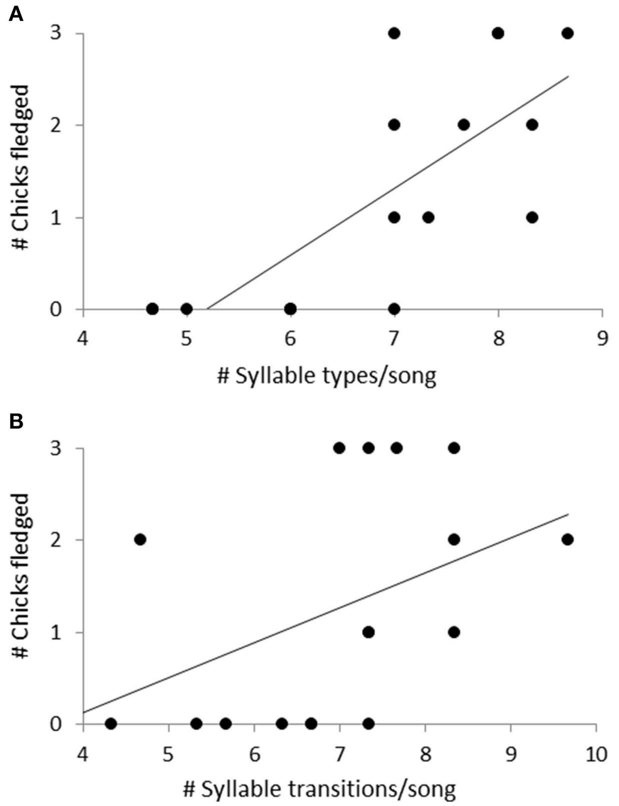

FIGURE 8 | The relationship between the number of chicks fledged and (A) the number of syllable types per song, and (B) the number of transitions between syllable types per song. Linear regression lines show direction of the relationship. These two parameters were associated with the PC2 scores of a PCA analysis looking at the effect of five song parameters on female reproductive success.

males were more likely to come to the nest when the female sang in the vicinity of the nest than when she did not (Halkin, 1997). In contrast, Kleindorfer et al. (2016) found in superb fairy wrens that female song in the nest incurs a significant cost in terms of increased risk of predation and they suggest this may provide a strong selective pressure for reduced female song during breeding. In our study, without manipulating female song rate or male provisioning rate, it is not possible to infer a causal relationship between these factors and chick fledging success. We cannot of course exclude the possibility of other correlated factors, for example, if female song rate is an honest signal of quality (Gil and Gahr, 2002), males may preferentially invest more in females with higher song rates. Furthermore, higher female song rates may secure better access to resources needed for chick-rearing; a possibility supported by our previous playback experiments that found high levels of aggression by territorial females to song playbacks of female neighbors (Brunton et al., 2008b). The lack of a significant effect of chick-provisioning rates and reproductive success was unexpected and is in part due to the strong effect of female song rate, and in part due to our imprecise measure of feeding rate: we were unable to determine quantity or quality of food being delivered to the chicks. Future experimental studies will be needed to test whether females may be increasing fledging success by soliciting higher male attentiveness at the nest.

For males, the literature provides mixed results for the relationship between song performance measures (repertoire, rate, and complexity) and fitness. Positive relationships between repertoire size and annual reproductive success have been demonstrated in great reed warblers, *Arcocephalus arundinaceus* (Catchpole, 1986; Hasselquist et al., 1996), song sparrows, *Melospiza melodia* (Hiebert et al., 1989; Reid et al., 2005), European starlings, (Eens et al., 1991), willow warblers, *Phylloscopus trochilus* (Gil and Slater, 2000), and zebra finches, *Taniopygia guttata* (Woodgate et al., 2012). In contrast, song is often not a reliable indicator of male quality and reproductive success [e.g., in great reed warblers (Forstmeier and Leisler, 2004), collared flycatchers, *Ficedula albicollis*, (Garamszegi et al., 2007), and rock sparrows, *Petronia petronia,* (Nemeth et al., 2012)]. The variation found in empirical studies of the relationship between male song quality and reproductive success, even within a single species, highlights the multimodal nature of sexually selected signals. Although varying song qualities may influence male song function and mating success, other traits such as plumage and behavior may provide stronger and/or alternative mechanisms for mate attraction, resulting in the absence of a link between song quality and reproductive success (Tobias et al., 2012).

Female Song and Social Selection

Because territorial song is so well-entrenched in the literature as a sexually selected male trait involved in competitive processes, many questions remain unanswered as to the nature of selection for song and other ornaments in females (Langmore, 1998; Riebel et al., 2005; Tobias et al., 2012). What are the selective drivers of female song performance and why should female song matter? In year-round territorial species such as bellbirds, where predators are limited, access to resources is likely to be the main predictor of reproductive success. Our study population is located on a conservation island where the main predators are native avian species (the diurnal, open country hunting Australasian harrier, *Circus approximans*, and the nocturnal morepork, *Ninox novaseelandiae*). The bellbird population is dense and likely to be at carrying capacity; therefore we expect that resources probably constrain reproduction and survival. Under such competitive conditions, aggressive social interactions are important and frequent. In general for birds, female reproductive success is not constrained by the number of mates, but by access to resources and parental investment (Bateman, 1948; Trivers, 1972).

If female song is linked to reproductive success but not via classical sexual selection (male attraction), then social selection may provide an alternative hypothesis (i.e., females use song and other competitive indicators to compete for ecological resources). Hence, female territorial song and traits involved in female competition may fit better within the concept of social selection (West-Eberhard, 1979, 1983; Amundsen, 2000; LeBas, 2006; Kraaijeveld et al., 2007; Rosvall, 2011; Tobias et al., 2011). Social selection (defined in West-Eberhard, 1979) focuses on the role of social interactions as drivers of selection, regardless of whether the interaction is sexual or non-sexual (Lyon and Montgomerie, 2012; Tobias et al., 2012). Although still not widely embraced and somewhat controversial, social selection predicts that the strength of social interactions may determine how aggressive traits such as vocalizations, coloration, weapons, and ornaments correlate with measures of reproductive success (McComb et al., 1994; Friedman et al., 2009; Cornwallis and Uller, 2010; Santana et al., 2012; Tobias et al., 2012; Morales et al., 2014).

Our study and several studies of superb fairy wrens (Kleindorfer et al., 2013, 2016; Cain et al., 2015) examine female song and reproductive success in free-living populations. In both bellbirds and superb fairy wrens, females sing complex songs year round at rates comparable to males and in the context of competition between females (Cooney and Cockburn, 1995; Brunton and Li, 2006; Brunton et al., 2008b; Kleindorfer et al., 2013). Aspects of the life history of these two species differ: superb fairy wrens are insectivores and cooperative breeders, whereas bellbirds are primarily nectarivores and exhibit social monogamy (Baillie et al., 2014). However, both species exhibit female dispersal and are non-migratory (Cockburn et al., 2008; Baillie et al., 2014). For these and other species with female song, it is likely that female song traits may convey information about competitive ability such as body condition and age could therefore influence the outcome of non-sexual social interactions and ultimately breeding success and survival.

It is unclear why male song rate does not predict the number of chicks fledged. We were unable to collect a sufficient number of male songs to be able to analyse male song structure and it is possible that song complexity rather than rate may be important in male song performance. Alternatively, part of the answer may be extra-pair paternity. Cope (2008) found high levels of extra-pair paternity in the Tiri bellbird populations and Wells et al. (2015) found high extra-pair paternity rates in a closely related New Zealand honeyeater, the tui (*Prosthemadera novaeseelandiae*). Taff et al. (2012) found that male plumage features were associated with within-pair mating success while song consistency was associated with extra-pair paternity in the common yellowthroat (*Geothlypis trichas*). Male song rates and song structure need to be explored further in order to understand how they relate to both mating success and parental investment in bellbirds.

Although our study was observational and we used a single measure of reproductive success, we found that female song rate and structure were good predictors of breeding success. Indeed, because the birds used in the behavioral observation dataset were independent of those used to analyze song structure, our findings are strengthened as analyzes of both datasets showed relationships between female song and breeding success.

The relationships reported here between female song structure, song rate, and reproductive success are based on correlational data. Future work should focus on experimental evidence to confirm the function of song performance in either increasing male provisioning or in social competition. Female song playbacks, clutch size manipulations, and genetic hereditability studies may help us tease apart correlative factors. In addition, how other phenotypic characteristics (e.g., size, body condition, and plumage), female age, and mate quality influence female song structure and rate needs to be explored.

CONCLUSIONS

Although this study is among the first to find a relationship between female song and reproductive success, there is a growing literature on the subject of female song and many species with territorial female song have yet to be studied. We used two simple approaches, observations of song rates and measures of song complexity to measure female song quality in relation to breeding success. We found the rate of spontaneous song by females during both incubation and the chick-rearing period was a good predictor for the number of chicks fledged. This was not the case for males. We also found that our two measures of female song complexity were positively correlated with higher numbers of fledglings. Overall our findings support the hypothesis that female song may be a signal of competitive ability (Cain and Ketterson, 2012; Tobias et al., 2012; Stockley and Campbell, 2013; Cain and Rosvall, 2014) but further work is required to experimentally test this relationship and to examine mechanisms (Cain and Ketterson, 2012). It is anticipated that as more studies examining the relationship between female song traits and reproductive success and survival are published, the extent of the connection between female song traits and female fitness will be revealed.

AUTHOR CONTRIBUTIONS

DB designed the study, developed the methodology, performed aspects of the analysis, and wrote the manuscript. MR collected the data, and assisted development of the methodology and managed the interns. AH performed the analyses and wrote sections of the paper.

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Female song rates in response to simulated intruder are positively related to reproductive success

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Bird song is well studied in males as a sexually selected behavior. However, although song is also common among females, it is infrequently examined and poorly understood. Research suggests that song is often used as a resource defense behavior and is important in female-female competition for limited resources, e.g., mates and territories. If so, song should be positively related to fitness and related to other resource defense behaviors, but this possibility has rarely been explored. Here we examine fitness estimates in relation to spontaneous song rates and song rates in response to a simulated intruder (playback), in the superb fairy-wren (*Malurus cyaneus*), a cooperatively breeding songbird. We also determine how song rates relate to other territorial defense behaviors. Song rate in response to playback, but not spontaneous song rate, was positively related to nest success and the number of fledglings produced by successful females. Further, response song rate was also correlated with other territorial defense behaviors (latency to respond and flights). This evidence supports the hypothesis that female song may be used in the context of female-female competition to improve access to limited reproductive resources, and suggests that song may provide direct fitness benefits.

Keywords: female aggression, female song, female competition, competitive traits, social selection, reproductive success

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INTRODUCTION

Territorial song is a classic example of a sexually selected trait in males, and usually functions in the contexts of male-male competition and female mate choice (Catchpole and Slater, 1995). However, females may also be territorial, and recent comparative work has shown that female song is both phylogenetically widespread and ancestral, suggesting that song may also serve critical functions in females (Price, 2009; Odom et al., 2014). Researchers have argued that female song may be an important signal used by females in intrasexual competition when critical resources are limited (e.g., social partners or territories; Langmore, 1998; Price et al., 2009; Odom et al., 2014). If so, variation in the expression of song should be positively related to resource acquisition and fitness. Further, if song is used in competitive scenarios, it should be positively associated with other defense behaviors, e.g., attacks. However, there are scant data addressing these possibilities (Eens and Pinxten, 1998; Langmore, 1998; Illes and Yunes-Jimenez, 2009; Illes, 2014).

Here, we begin to address these questions by examining the relationships between female fitness, song, and other territorial defense behaviors in the superb fairy-wren (*Malurus cyaneus*). Superb fairy-wrens are socially monogamous, though >70% of young are sired by extra-group males in our study population (Dunn et al., 2001). They also are cooperative breeders: sons from previous broods may remain on the natal territory as subordinate helpers (Cockburn et al., 2003). However, daughters disperse and must acquire and defend a territory or die; there are no floater females (Cockburn et al., 2008). Multiple lines of evidence suggest that female fairy-wrens appear to use song to defend breeding resources. Females sing year-round, and at rates similar to males (excluding the dawn chorus; Cooney and Cockburn, 1995). Female song rates peak during the transition from winter to pre-breeding, during initial territory establishment (Cooney and Cockburn, 1995), and song rates increase slightly across date within the breeding season (Cain and Langmore, 2015). Further, females, but not males, increase song rates in response to a simulated female intruder, i.e., playback (Cain and Langmore, 2015).

To test the hypothesis that song rate is important in territory defense and has important consequences for female fitness we examined the relationship between reproductive success and song in two contexts: spontaneous song rates and song in response to a simulated female intruder (playback). We also examined how song rates relate to other territorial defense behaviors (flights and latency to respond). We predicted that if female song rate is important for acquiring and maintaining a quality breeding territory or other reproductive resources, then reproductive success would be positively related to song rates. Further, if female song functions in territorial defense, we predicted that song rates in response to playback would be positively related to other response behaviors.

METHODS

Study System

This study was conducted in the Australian National Botanic Gardens (BG), ACT, Australia (35°16′S, 149°06′E). All adults were uniquely color-banded, and group composition and all nesting attempts were monitored from 27 August to 2 March. The first egg was laid on 16 September and the last brood fledged on 5 February (maximum of three successful broods per season). Previous research found age differences in success between first-year females and older females (Cockburn et al., 2008), thus we classified females as young (first year only, $n = 8$) or old (beyond first year, $n = 21$) using previous banding records. The same study found that high quality territories accumulate more subordinates, and thus are more likely to be occupied by group breeding females, so we coded females as group or pair-breeding.

Understanding the selective advantages of behavior requires the use of quality fitness estimates, and the benefits of female competition are often poorly captured by immediate fitness proxies, e.g., number of eggs (Clutton-Brock, 2009; Tobias et al., 2012; Cain and Rosvall, 2014). We use three fitness proxies that integrate longer time periods and better estimates of realized fitness. First, nest success is a critical selective period for passerines and is perhaps the most important fitness component for breeding females (Martin, 1995, 2015). Females were categorized as successful if they fledged at least one offspring ($n =$ 19 successful, 10 unsuccessful). The number of fledglings may provide nuanced information about relative fitness; therefore we also examined total annual fledgling production. Finally, because predation can also be high just after fledging, we also examine relationships with number of independent young, previously defined as 4 weeks post fledgling (Cockburn et al., 2008).

Song Rates and Territorial Behaviors

We quantified female song rates using a standardized behavioral paradigm that allowed us to determine both spontaneous song rates, and song rates in response to a simulated intruder (Cain and Langmore, 2015). Spontaneous song rates (songs per min) were quantified by passively observing focal females for 10 min and counting songs produced. After the observation period we simulated a foreign female intruding on the resident female's territory using playback of unfamiliar female song and quantified female response: number of songs, flights greater than 1 m, and latency to respond with a flight or song. We constructed 18 unique stimuli from female fairy-wrens at least 5 km away. Each playback consisted of two unique songs taken from the same female repeated six times in alternate order (for more details see Cain and Langmore, 2015). All focal females were paired, but did not yet have eggs in the nest. Territorial behavior and pairing begins in August (Cockburn et al., 2009). Trials occurred between 15 September and 15 November 2012, between 7:00 and 13:00. The Australian National University Animal Experimental Ethics Committee (protocol A2012/54) and the Australian Capital Territory Municipal Services (license LT 2012559) approved all procedures.

Statistical Analysis

To test how spontaneous and response song rates relate to other territorial defense behaviors, we determined the correlation coefficients between pre-trial song-rate, response song rate, number of flights, closest approach, and latency to respond. Because superb fairy-wren song rates are related to date (Cain and Langmore, 2015), we regressed both measures of song rate on date to calculate date-adjusted song rates for further analysis, regressions for spontaneous, and response song rates were done separately. To examine the relationship between fitness estimates and female response, we built three generalized linear models, using different fitness estimates as the dependent variable and spontaneous song rates (date adjusted), response song rates (date adjusted), age, and age by song rate interactions as potential predictors variables. The first model examined the relationships between nest success (binomial, successful, or failed); the second and third models focused on the subset of females that produced at least one successful nest, and examined the relationships between number of fledglings and the number of independent young (normal error distributions). We used stepwise backward procedures to remove non-significant variables ($P > 0.1$ to remove), starting with interactions.

RESULTS

Song and Territorial Behavior

Behaviors in response to playback of novel female song (songs, flights, latency to response) were significantly inter-correlated;

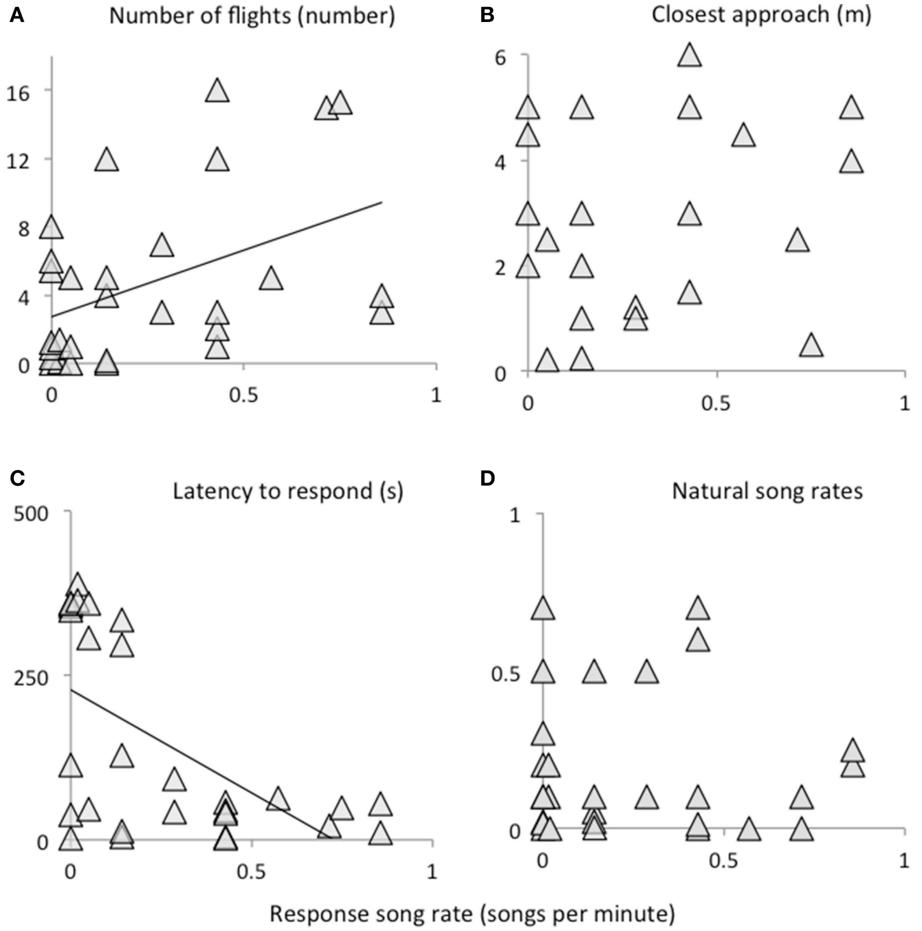

FIGURE 1 | Scatterplots illustrating the relationships between response song rate and defense behaviors: (A) number of flights, (B) closest approach, (C) latency to respond, and (D) natural song rate.

i.e., birds that responded strongly in one measure tended to respond strongly in other measures (**Figure 1**, **Table 1**). Closest approach was unrelated to song rates, but negatively related to the number of flights (birds that approached closer also had more dives), however the relationship was a trend. Pre-trial song rates were unrelated to any response variables during the playback trial period (all $P < 0.15$).

Song Rates and Reproductive Success

Individuals with higher response song rates were more likely to have at least one successful nesting attempt (**Figure 2**, **Table 2**). Among successful females, the total number of fledglings produced across the breeding season was also positively related to response song rate, although it was not statistically significant (**Figure 3**, **Table 2**). Spontaneous song rates were unrelated to any fitness estimate (all $P > 0.15$).

TABLE 1 | Correlation matrix for pre-trial song rates, and behaviors in response to simulated intrusion using female playback.

	Trial song-rate	Flights	Latency to respond	Closest approach
Pretrial song-rate	−0.019 (0.92)	0.07 (0.71)	0.20 (0.30)	−0.012 (0.95)
Trial song-rate	–	**0.45 (0.015)**	**−0.59 (0.0008)**	0.20 (0.37)
Flights		–	**−0.69 (<0.0001)**	*−0.38 (0.084)*
Latency to respond			–	0.11 (0.64)

Values are Spearman's correlations (r) with p-values provided in parenthesis. Bold values are significant (p < 0.05); italicized values are trends (p < 0.10). Negative values for closest approach and latency indicate a more robust response.

DISCUSSION

Our results show that female song is a common element of territorial response to a simulated intruder (playback) and reveal a positive relationship between territorial song rates (response) and two critical components of fitness, nest success, and number of fledglings. However, among successful females, individual differences in response song rates were unrelated to the number of young that survived 4 weeks after fledging, and spontaneous song-rates were unrelated to any fitness estimate.

We found strong relationships between response song rates and other territorial behaviors, replicating earlier findings

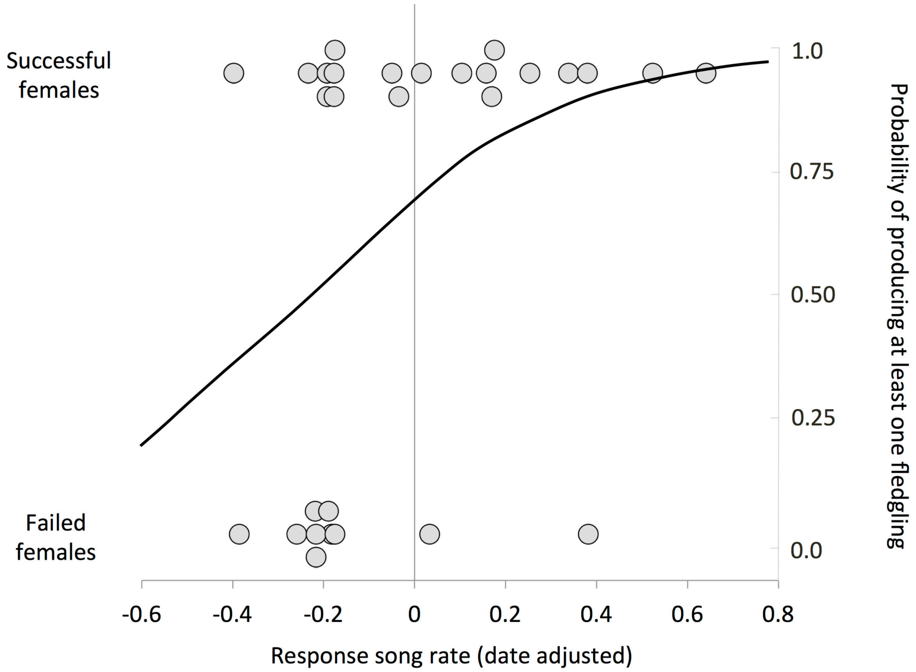

FIGURE 2 | Relationship between female response song rate and nest success. Response song rates were plotted according to whether the female was successful (produced at least one fledgling) or failed in all attempts; overlapping points are jittered for clarity. Line is a logistic regression estimating the probability that a female with a given response score would be successful.

TABLE 2 | Results from generalized linear models relating fitness estimates to song rates.

Fitness measure	Effects	Estimate ± SE	*P*-value	Final model?
Fledging production (failure/success) $X^2 = 4.3$, $n = 29$, $P = 0.039$ Binomial errors	**Response score (Date adj.)**	**3.6 ± 2.0**	**0.039**	**Yes**
	Age (old/young)	0.57 ± 0.48	0.23	No
	Spontaneous song rate	0.88 ± 2.2	0.68	No
	Age × Response	−7.7 ± 10.0	0.20	No
	Age × Spontaneous song rate	0.82 ± 2.8	0.76	No
Number of fledglings, successful females only $X^2 = 8.6$, $n = 18$, $P = 0.013$ Normal errors	*Response score (Date adj.)*	*2.4 ± 1.2*	*0.061*	*Yes*
	Age (old/young)	**0.98 ± 0.46**	**0.045**	**Yes**
	Spontaneous song rate	1.0 ± 1.6	0.53	No
	Age × Response	−1.6 ± 3.0	0.59	No
	Age × Spontaneous song rate	−5.1 ± 31	0.89	No
Number of independent young, successful females only $X^2 =$, $n = 18$, $P = 0.074$ Normal errors	Response score (Date adj.)	1.5 ± 1.2	0.24	No
	Age (old/young)	*0.85 ± 0.46*	*0.074*	*Yes*
	Spontaneous song rate	0.87 ± 1.6	0.58	No
	Age × Response	5.0 ± 43	0.91	No
	Age × Spontaneous song rate	−1.7 ± 2.1	0.44	No

Results are from GLM models with appropriate error distributions, statistically significant variables are in bold (P < 0.05), trends in italics (P < 0.10). We used stepwise backward procedures to remove non-significant variables (P > 0.1 to remove), starting with interactions. Values for excluded variables provided from the step before the variable was removed.

(Cooney and Cockburn, 1995). These findings echo other research on female song. In European starlings, spontaneous song rate was positively related to number of aggressive interactions (Pavlova et al., 2007). In New Zealand bellbirds (*Anthornis melanura*), females counter-sing more to neighbors than strangers and also approach the speaker more closely for neighbors (Brunton et al., 2008), and in stripe-headed sparrows (*Aimophila r. ruficauda*) singing behavior in response to female playback showed similar patterns to approaches, latencies, and flights (Illes and Yunes-Jimenez, 2009). Vocalizations in female

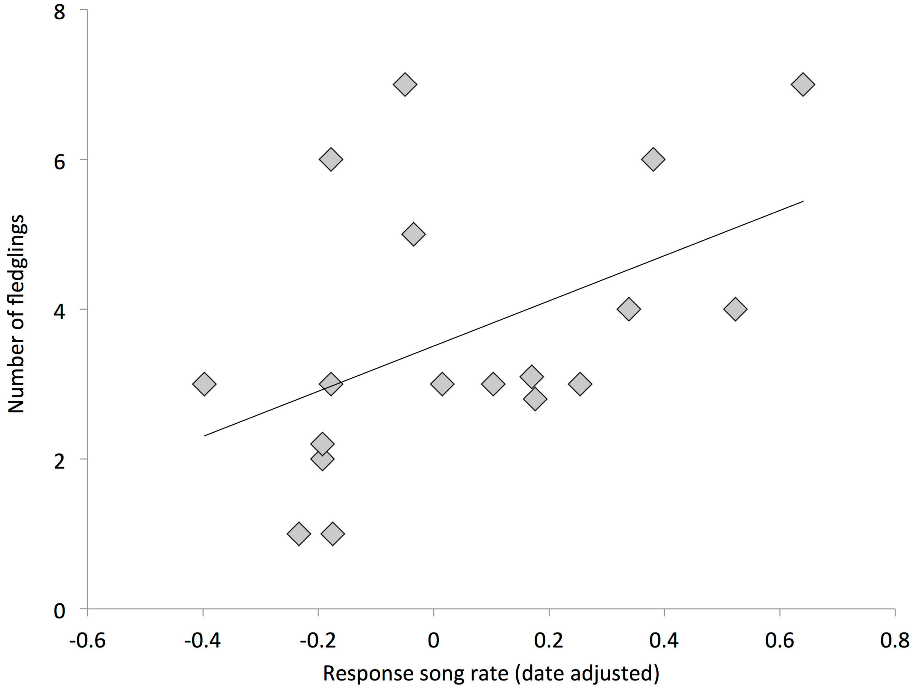

FIGURE 3 | Relationship between female response song rates and total fledglings produced (among successful females); overlapping points are jittered for clarity. Line is from a linear regression.

dunnocks (*Prunella modularis*) were associated with physical attacks (Langmore and Davies, 1997). Female purple-crowned fairy-wren (*Malurus coronatus*), increased song rates and rapidly approached the speaker in response to playback (Hall and Peters, 2008). Taken together, these results suggest that song is an important component of resource defense behavior for female songbirds (Langmore, 1998). However, it is still unclear whether any aspect of song deters or repels potential rivals, or whether any aspect of song is directly related to resource acquisition (e.g., territory quality). This possibility is an important avenue for future research.

Nest success sets an upper limit on female reproductive success (Martin, 1995, 2015). Thus, the positive relationship between nest success and female response song rates indicates an important fitness advantage. Further, the positive relationship between fledgling number and response song rates suggests that even among successful females, females that sing more frequently in response to novel female song experience a continued advantage. However, caution is warranted in interpreting these results given that this pattern had eroded by 4 weeks post fledgling, the approximate time of independence (Cockburn et al., 2008). This lack of relationship may be a statistical limitation, i.e., low detection power due to small sample. However, the lack of relationship may also be a product of real costs for strong responders, or due to stochastic processes. For instance, predation pressure can be very strong in the first week after fledging, which might undermine a portion of the advantages strongly responding females experienced. This possibility is supported by research in other passerines that reported negative relationships between some measure of maternal care and aggression (Dunn et al., 2001; Rosvall, 2011a; Cain and Ketterson, 2013).

There are a number of potential mechanisms that may underlie the positive relationship between response song rates and two of our critical fitness estimates, nest success, and fledgling number. First, response song rates may reflect individual competitive ability or quality, i.e., females capable of a high response song rate may be more likely to acquire high-quality territories (Cockburn et al., 2003; Rosvall, 2008; Cain and Ketterson, 2012). A second possibility is that females occupying high quality territories defend them more robustly than females on low quality territories (Enquist and Leimar, 1987; Cooney and Cockburn, 1995). In either case, we would expect territory quality to be positively related to response song rates. We were unable to test this directly, however, past research in this population of fairy-wrens found that territories with high nest success accumulate more subordinates, suggesting that high-quality territories are more likely to have subordinates (Cooney and Cockburn, 1995; Cockburn et al., 2008). Thus, if response song rates predicted territory quality, or if territory quality drove female response song rates, we would expect that response song rates would be higher in group-breeding females. However, previous research found no difference in response song rates scores between group and pair-breeding females (Cain and Langmore, 2015). However, this is a coarse measure and differences in territory quality may yet be an important mechanism underlying the positive relationship within pair-breeding females. Thus, this is an important direction for future research.

Finally, the positive relationship between response song rates and reproductive success might occur because female response song rate is related to a third, unmeasured variable that positively influences reproductive success. For example, in other female songbirds, response to a simulated intruder has been positively related to predator defense behavior (Clutton-Brock, 2009; Cain et al., 2011; Tobias et al., 2012; Cain and Rosvall, 2014), body size (Langston et al., 1990; Martin, 1995; Cain and Ketterson, 2012), and maternal care (Rosvall, 2011a; Cain and Ketterson, 2013). Further, a robust response might also be a reflection of overall activity level. Female aggression and territorial defense behaviors have been related to testosterone levels in other passerines and high testosterone levels can increase activity (Langmore et al., 2002; Zysling et al., 2006; Cain and Ketterson, 2012; Rosvall, 2013). Further research is needed before we can conclude whether selection is acting directly on female song rates or some other aspect of female phenotype.

In contrast to the positive relationships between response song rates to a playback of a novel female and reproductive success, we found no relationships between spontaneous song rates and fitness estimates. This lack of relationship may be a by-product of our experimental protocol; the observation period was 10 min, which may not be sufficient to capture important variation in spontaneous song rates. Alternatively, it may be that variation in spontaneous song rates is less important for defending a territory, or is unrelated to the quality of the territory or female. Support for this possibility comes from previous research on female fairy-wren song, which found that spontaneous song rates are highest when a female has recently established a new territory, suggesting song serves a territorial function, but is not maintained at a high level once ownership is established (Cooney and Cockburn, 1995). A final possibility is that it is some aspect of the song itself, rather than the number of songs that is important. Research in female European starlings (*Sturnus vulgaris*) has shown that song complexity and performance are repeatable, and that repertoire changes with age, suggesting that song traits may be quality indicators (Pavlova et al., 2010). Similarly, older female alpine acceptors (*Prunella collaris*) sang more complex songs, and laid larger clutches (Langmore et al., 1996).

CONCLUSION

Females in a variety of species appear to use ornaments, weapons, and same-sex aggression to attract and defend mates (Amundsen, 2000; Weiss, 2006), or compete with other females for critical reproductive resources (Robinson and Kruuk, 2007; Rosvall, 2008; Watson and Simmons, 2010). The results presented here join a growing body of work that reports positive relationships between fitness estimates and competitive trait expression in females (Rosvall, 2008; Sinn et al., 2008; Watson and Simmons, 2010; Cain and Ketterson, 2012, 2013), and suggests that the persistence of female competitive traits are in many cases the product of direct selection favoring exaggerated traits when female-female competition is strong (Rosvall, 2011b; Tobias et al., 2012; Stockley and Campbell, 2013; Cain and Rosvall, 2014).

To our knowledge, this is the first study to examine the relationship between fitness and female song. Future work should determine the mechanisms of this relationship and to examine how song rates relate to other fitness components, which would add critical insights regarding the costs and benefits of trait expression (Cain and Rosvall, 2014). Finally, examining the relationship between female competitive traits and fitness in different contexts, such as when resource availability differs, will be essential to our understanding how selection shapes trait expression (Cain and Rosvall, 2014).

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Song in a Social and Sexual Context: Vocalizations Signal Identity and Rank in Both Sexes of a Cooperative Breeder

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In most songbirds, both sexes produce calls, or short vocalizations used to coordinate behaviors and maintain social cohesion. In contrast, songs are longer, more elaborate vocalizations typically only produced by males in behavioral contexts shaped by sexual selection operating through female choice. However, both males and females sing in many cooperatively breeding species, including the superb starling (*Lamprotornis superbus*). In this species, both sexes produce songs and calls composed of sequences of temporally discrete elements called motifs. Calls signal social group and individual identity, but the function of songs is currently unknown. Because superb starlings often sing in groups, song could be used not only in a sexual context, but also to signal identity and rank within the separate dominance hierarchies observed in males and females. To determine whether songs are used in mate attraction (sexually selected) and/or to influence social rank (socially selected), we compared song diversity with three potential indicators of fitness and dominance: social status, the number of seasons spent breeding, and age. We found that age is correlated with song diversity in both males and females, suggesting that (1) these signals serve similar purposes in both sexes, and (2) song diversity is likely the result of selection by both mutual mate choice and social competition. To test whether songs carry a signal of individuality, we applied spectrogram dynamic time warping to measure pairwise similarity among song motifs, and then calculated motif similarity within and between individuals. We found that motif similarity is higher within individuals than between individuals, suggesting that songs signal individual identity, which may help to establish social rank. These results are consistent with the hypothesis that superb starling vocal behavior in each sex is shaped by both social and sexual selection. Additionally, because call motifs are also used in songs, our data suggest that at least some vocal building blocks have evolved to convey multiple signaler traits and to facilitate complex social and sexual interactions in different contexts.

Keywords: female song, sexual selection, social selection, cooperative breeding, *Lamprotornis superbus*

INTRODUCTION

Bird song has traditionally been viewed either as a male ornament used to attract females or an armament used to defend territories against other males (Darwin, 1859). Under the traditional model of sexual selection (Darwin, 1871), song was thought to be an example of male trait elaboration resulting from differential selection pressures between the sexes due to female choice (Andersson, 1994). Female choice remains a common explanation for male song in temperate-dwelling species that have socially monogamous mating systems (Catchpole and Slater, 2008). However, recent studies have shown that a large proportion of avian species exhibit female song (Langmore, 1998; Garamszegi et al., 2007; Odom et al., 2014). For example, several studies have shown that female song frequently occurs in tropical species that occupy year-round territories (Morton, 1996; Langmore, 1998; Hall, 2004; Slater and Mann, 2004; Price, 2009; Price et al., 2009; Tobias et al., 2011), as well as in species where females may use song for territory defense (Cooney and Cockburn, 1995). In species where competition among females is high, it has recently been suggested that elaborate female traits are under sexual selection much in the same way as they are in males: acting through male mate choice and female-female competition (Clutton-Brock, 2007, 2009; Rubenstein and Lovette, 2009; Rubenstein, 2012b). There is also increasingly strong support for the idea that ornamentation in females can be influenced by social selection, or social competition for ecological resources that indirectly lead to an increased likelihood of reproducing (Crook, 1972; West-Eberhard, 1979, 1983; Lyon and Montgomerie, 2012; Tobias et al., 2012). Thus, there has been a renewed interest in female ornamentation and the recognition that we must develop models of selection that not only account for this mutual trait elaboration (Clutton-Brock, 2007; Tobias et al., 2011; Rubenstein, 2012a), but also reframe sexual selection theory to be more universally applicable to trait elaboration in both sexes (Jones and Ratterman, 2009; Price, 2015).

Among highly social birds, a suite of selective forces may drive patterns of trait elaboration in both sexes. In cooperatively breeding societies, for example, social selection for shared resources as well as sexual selection for mates may lead to ornamentation in both males and females because there is typically strong competition for limited breeding positions in each sex (Rubenstein and Lovette, 2009). Additionally, in societies where animals live in stable groups, dominance hierarchies often emerge (Clutton-Brock and Huchard, 2013), which could lead to selection for traits that aid in establishing social rank (reviewed in Tibbetts and Dale, 2007). In some cases, different features of a single trait may be shaped simultaneously by both sexual and social selection, such that the trait serves both to attract mates and to aid in competition for ecological resources and/or social rank. For example, in cooperatively breeding mockingbirds, song repertoire size appears to be driven by sexual selection operating though female choice (Howard, 1974), whereas song consistency has been shown to function in both a social and sexual context, signaling age, social dominance, and reproductive success in males (Botero et al., 2009). Similarly, in cooperatively breeding Malurids, the strength of sexual selection is correlated with singing rates in males of several species, but syllable diversity is determined by environmental factors correlated with latitude (Greig et al., 2013). Finally, simple vocalizations (e.g., calls) are under strong selection to signal individual, kin, or group identity in many cooperatively breeding birds (Payne et al., 1988; Price, 1998; McDonald and Wright, 2011), suggesting that vocal signals may also be shaped by the need to facilitate cooperation and competition in socially complex species. Signaling identity can help to establish rank or maintain dominance hierarchies (Barnard and Burk, 1979; Pagel and Dawkins, 1997), and the need to signal individual identity may therefore play a role in shaping songs in social species. Although it is widely accepted that song is constrained by multiple traits (Gil and Gahr, 2002), few studies have examined how sexual and social selection—including the need to signal identity—shape song evolution and the extent to which this occurs in both males and females. Furthermore, to our knowledge, no previous studies have examined calls and songs together to compare the relative strength of selection on these different types of vocal signals.

Here we investigate the calls and songs of male and female cooperatively breeding superb starlings (*Lamprotornis superbus*) to explore how sexual selection to attract or gain access to mates, as well as social selection to establish dominance rank, may influence trait evolution. Superb starlings are plural cooperative breeders that live in large social groups of up to 30 or more birds that can include as many as six breeding pairs (Rubenstein, 2016). Many non-breeders serve as helpers at the nest, and there is high intrasexual competition in both males and females for limited breeding positions (Rubenstein, 2007a). As in other African starlings that breed cooperatively, superb starlings show reduced sexual dimorphism in body size and plumage (Rubenstein and Lovette, 2009), and Bateman gradients are similar in males and females, further supporting the idea that both sexes are under strong sexual selection (Apakupakul and Rubenstein, 2015). This lack of sexual dimorphism extends to song as well, with both males and females producing similarly complex songs (Pilowsky and Rubenstein, 2013). Superb starling songs include a large number of unique motifs, or single notes that are arranged in various combinations (Pilowsky and Rubenstein, 2013). Starlings also produce short, relatively simple vocalizations (i.e., four or five motifs long) when flying over group territories. These flight calls have been shown to carry a strong signal of individual identity and social group membership, and are therefore thought to function in recognition (Keen et al., 2013). Interestingly, all of the motifs used in flight calls (hereafter, "calls") also appear in songs, meaning that starlings take these basic vocal components and add many more song-specific motifs to create elaborate vocal displays when singing.

Superb starling breeders have been shown to produce more unique motifs in their songs than non-breeders (Pilowsky and Rubenstein, 2013), but exactly how song functions in a social and sexual context in this species remains unclear. We hypothesize that song elaboration is shaped by mutual mate choice (i.e., sexual selection in both sexes), and predict that song diversity is correlated with reproductive success in both males and females. Furthermore, because songs include many of the motifs used in flight calls, we hypothesize that song also functions in recognition, which may be important for establishing dominance rank. We predict that a signal of individual identity is embedded within a song, just as it is within a call. Consequently, we expect that song is a complex signal shaped by both sexual and social selection to signal fitness, social rank, and identity. To test these predictions, we analyzed song and call recordings collected from male and female superb starlings to determine (1) if song diversity is correlated with total lifetime breeding opportunities, age, or status, (2) if a signal of individual identity is present in songs and whether it is stronger than that in calls, and (3) if song diversity and individuality are equivalent in males and females.

METHODS

Study Population

All data were collected from a free-living population of superb starlings at the Mpala Research Centre, Laikipia, Kenya between May and July 2008–2011. This population has been studied continuously since 2001, and individuals have been marked with a unique combination of colored leg bands and a metal leg ring with an identification number (Rubenstein, 2007a). The study population includes nine social groups that maintain year-round territories. Male superb starlings are typically philopatric, whereas females immigrate after reaching maturity (Rubenstein, 2007a). However, within-group relatedness among males is lower than expected, as nearly half of all male breeders may be immigrants (Pollack and Rubenstein, 2015; Rubenstein, 2016). Additionally, relatedness among females is higher than expected, as immigrant females recruit sisters to their new groups (Pollack and Rubenstein, 2015). Thus, kin structure is present in both sexes within a group, though higher than expected in females and lower than expected in males (Rubenstein, 2016). All field work for this study was approved by Columbia University's Institutional Animal Care and Use Committee (#AC-AAAB1128).

Song Data

Audio recordings of songs and calls were collected as part of previous studies (Keen et al., 2013; Meliza et al., 2013; Pilowsky and Rubenstein, 2013) using a Sennheiser ME66 directional microphone (Sennheiser Electronic, Old Lyme, CT) and a Marantz PMD661 digital recorder (Marantz, Mahwah, NJ). Audio files were saved as 16-bit, 44 kHz wav files and were automatically time-stamped upon recording. Focal birds were identified using a spotting scope, and age, breeding status, and the total number of seasons spent breeding were obtained from behavioral observations and long-term census records (Pollack and Rubenstein, 2015; Rubenstein, 2016).

Songs, which we defined as vocalizations lasting more than 5 s, were recorded from 28 individuals (16 males and 12 females) in five social groups. In contrast, bouts of flight calls were much shorter and typically lasted between 0.5 and 2 s. Songs were collected from two individuals in 2008 and 26 individuals in 2011; songs were not collected from the same individual in multiple years. We showed previously that at least 8 min of song are necessary to assess a superb starling's repertoire size (Pilowsky and Rubenstein, 2013). Therefore, we collected 8.8 ± 0.2 min (mean ± SD) of song from the 28 sampled individuals, and 26 of these individuals had over 8 min of song. Superb starlings sing only when perched, and during singing do not perform other behaviors except for occasional preening (Pilowsky and Rubenstein, 2013). All recordings were divided into individual wav files each containing a single song motif (*sensu* Pilowsky and Rubenstein, 2013), using Raven Pro 1.2 (Cornell Lab of Ornithology, Ithaca, NY, U.S.A.). Motifs were identified as single notes that are visible as continuous tonal elements in spectrograms and can be separated from adjacent motifs by the absence of any power between 1 and 12 kHz for at least 40 ms (see **Figure 1**). Motif categories were created during a previous study of song in this population (Pilowsky and Rubenstein, 2013; see data supplement for motif category key). This yielded a dataset of 20,423 song motifs (12,797 from males and 7626 from females), and 729 ± 249 motifs (mean ± SD) per individual. All song motifs were then manually labeled by J.A.P. as belonging to one of 87 distinct motif classes.

Flight call recordings were collected during 2008–2010 from 109 individuals (56 males and 53 females), including at least five birds in each of the nine social groups. Call recordings were divided into motifs in the same manner as songs, and were classified into 18 unique classes, all of which also appear in songs. After excluding recordings of poor quality or those in which the focal bird was unidentifiable, the final dataset contained 1936 call motifs (1095 from males and 841 from females), with 39 ± 19 motifs (mean ± SD) motifs per individual. Spectrograms depicting songs and calls are shown in **Figure 1**.

When recording both flight calls and songs, the microphone was always placed within 5–10 m of the focal bird, and the gain on the digital recorder was adjusted to ensure that the recording maximized the dynamic range of the digitizer but did not clip. As an additional means of accounting for differences in recording distance, the amplitude of all wav files of single motifs were normalized before any sound analysis was performed.

Song Diversity Analyses

We calculated within-bird song motif diversity for the 28 individuals from which we had song recordings using the Shannon Diversity Index (H') (Shannon and Weaver, 1949) of the 87 manually-assigned motif labels observed in the larger population, yielding a single measure of song diversity for each individual. The Shannon diversity index was selected because it accounts for both richness (i.e., the number of different types of motifs in an individual's song) as well as evenness (i.e., their relative abundances), and has been shown to be the best metric for combining these components into a single estimate of diversity (Stirling and Wilsey, 2001). We used linear mixed-effects models to test whether age, status at time of recording, proportion of seasons spent breeding, sex, and social group were predictors of song diversity. Based on model comparisons using Akaike's information criterion (AICc) (summarized in Table S1), we excluded interaction terms from the model. All of the predictors showed a low degree of multicollinearity

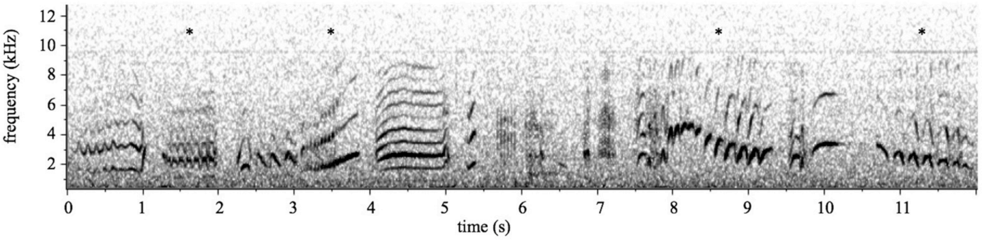

FIGURE 1 | Spectrogram of superb starling song. Superb starling song is a complex signal comprising over 80 unique motifs used in various combinations. In contrast, superb starling calls are relatively simple combinations of up to 20 possible motifs, though a single call bout typically contains only 4–5 motifs. Notably, all of the motifs used in calls are also found in songs. This 11-s spectrogram of superb starling song includes several motifs that are also used in flight calls, indicated here by asterisks.

(all VIF < 2.2). In our model, social group was used as a random effect; all other variables were included as fixed effects. Status at time of recording was recorded as "breeder" or "non-breeder," which was determined through nest observations during the season in which songs were recorded. The proportion of seasons spent breeding was calculated as the total number of seasons during which an individual held "breeder" status, divided by the total number of seasons in which they were alive and capable of holding a breeding position (i.e., older than 1 year of age). We used this measurement rather than lifetime reproductive success (i.e., total number of offspring fledged) because high nest predation rates and unpredictable breeding conditions in this population make this an unreliable indicator of the number of breeding opportunities an individual obtained. Moreover, we know from previous work in this system that the number of seasons breeding is the strongest predictor of lifetime reproductive success (Apakupakul and Rubenstein, 2015). Thus, our selected predictor variables (i.e., age, current status, proportion of seasons breeding) are all believed to be correlated with fitness and dominance status in superb starlings.

Song Similarity Analyses

To determine if songs carry a signal of individual or group identity, we used dynamic time warping (hereafter DTW) (Vintsyuk, 1971; Kogan and Margoliash, 1998) to compare spectrograms of individual song motifs to one another (See Table S2 for spectrograms of all motifs in our dataset). DTW quantifies the similarity of two spectrograms by compressing or expanding the reference spectrogram on the time axis in order to find the best fit (Vintsyuk, 1971). This method is less sensitive to background noise than spectrographic cross-correlation and produces similarity measures that more closely match human assessments of similarity between spectrograms of superb starling vocalizations (Meliza et al., 2013). The performance and repeatability of the DTW algorithm on call motifs in superb starlings was examined previously (Meliza et al., 2013); we also examined the algorithm's concordance with song motif categories and found that average similarity scores within categories (mean $\pm$ SE $= 2.57 \pm 0.002$) were significantly greater than scores between song motif categories (mean $\pm$ SE $= 2.46 \pm 0.0004$; t-test: $t = -60.5$, $p < 0.001$).

We calculated similarity scores for all pairs of call and song motifs in the dataset using the pairwise distance metric output by the DTW analysis (*sensu* Meliza et al., 2013). We then identified the best match for each motif within the repertoire of every bird in the dataset, including the individual that sang the reference motif (Keen et al., 2013). This best match score is high if there is a close match with a motif in the target bird's repertoire, but low if there is not. Best match scores were then grouped by whether the target bird was (1) the individual that sang the reference motif, (2) in the same social group, or (3) in a different social group. Call and song motifs were analyzed separately. We used a linear mixed-effects model (LMM) to test whether mean best match scores (log transformed for normality) depended upon social relationship (i.e., same bird, same group, or different group), with random effects included for the year the recording was collected, the sex, the social group of the birds whose motifs were being compared, and the reference motif used. The number of motifs tested for each comparison bird was also included as a covariate to account for differences in the number of samples per individual and the increased probability of finding a good match with larger numbers of comparisons.

To test whether within-bird similarity was higher within songs or calls, we used a Welch two sample t-test to compare mean within-bird song best match scores ($N = 28$) to mean within-bird call best match scores ($N = 89$). Similarly, we used a Welch two-sample t-test to compare whether mean within-bird call and song similarity was higher in males or females, using separate tests for calls and songs. In both cases Welch t-tests were used to account for unequal variances.

All statistical tests were conducted in R (R Development Core Team, 2015); mixed-effects models were fit using *lme4* (Bates et al., 2015), and the significance of fixed effects was evaluated using Satterthwaite approximations to estimate effective degrees of freedom. *Post-hoc* comparisons used Tukey's tests to evaluate significance. Although this technique is nearly identical to that used in Keen et al. (2013) to analyze call motifs, song motifs were compared here using DTW of spectrograms rather than pitch traces. Pitch-based DTW is more sensitive than spectral DTW because it effectively eliminates background noise, though the results are qualitatively similar (Meliza et al., 2013).

RESULTS

Song Diversity Is Correlated with Age and Breeding Experience

Song diversity increased with age (LMM: $t = 2.37$, $p = 0.028$; **Figure 2A**), but decreased with the proportion of seasons spent breeding ($t = -2.49$, $p = 0.021$). Although **Figure 2B** appears to show a positive relationship between song diversity and breeding experience, once the correlation with age is taken into account, the data indicate that more successful breeders tend to have less diverse song repertoires. However, there was a trend for breeders at the time of the recording to have more song diversity than helpers (LMM: $t = 2.01$, $p = 0.056$; **Figure 2C**). Males and females sang equally diverse songs (LMM: $t = 0.40$, $p = 0.69$; **Figure 2D**), and there was little variation among groups in song diversity relative to variation within groups (SD among groups = 0.057; residual SD = 0.15). These results are summarized in **Table 1**.

Songs Carry a Signal of Individual Identity but Not Social Group

Song motifs from the same bird's repertoire were significantly more similar to each other than to song motifs of birds in the same social group (LMM Tukey test: $t = 47.4$, $p < 0.001$; **Figure 3A**), as well as song motifs of birds in other social groups ($t = 5.71$, $p < 0.001$; **Figure 3A**). However, song motifs were not more likely to be similar to those of other birds in the same social group than to song motifs of those in other groups ($t = 1.61$, $p = 0.21$; **Figure 3A**).

As with song motifs, call motifs from the same bird's song repertoire were significantly more similar to each other than to same-group birds (LMM Tukey test: $t = 34.5$, $p < 0.001$; **Figure 3B**) as well as to extra-group birds ($t = 21.676$, $p < 0.001$; **Figure 3B**). Unlike song, however, call motifs were more similar to calls from other individuals in the same social group than to those of birds in the larger population ($t = 4.93$, $p < 0.05$; **Figure 3B**).

Differences Between Signal Types and Sexes

Within-bird similarity in songs was significantly higher than in calls (Welch two sample t-test: $t = -13.96$, $p < 0.001$; **Figure 3C**). In both songs and calls, males and females did not have significantly different levels of within-bird motif similarity (t-test: song, $t = -0.75$, $p = 0.46$; call, $t = -0.57$, $p = 0.57$; **Figure 3D**).

DISCUSSION

Our results show that older birds sing more diverse songs, consistent with the hypothesis that song complexity is a signal of current status within superb starling social groups. Interestingly, individuals with more breeding experience tended to sing less diverse songs than individuals of the same age, suggesting that additional factors may influence the development of more complex songs. Moreover, song motif similarity was significantly higher when comparing motifs produced by the same individual than when comparing motifs produced by different birds. Together, these results suggest that superb starling song is likely to signals status and individual identity, and that song complexity may stabilize or decrease in older individuals once breeding positions are obtained. The observed equivalent degree of song diversity in males and females indicates that selection for elaborate songs may be equally strong in both sexes. This result is consistent with reproductive success data from this population showing that males and females are both likely to be under strong sexual selection for access to mates (Apakupakul and Rubenstein, 2015).

In studies of species with mating systems driven by female choice, older mates have often been shown to be preferred by females (Andersson, 1994; Kokko and Lindström, 1996; Kokko, 1997). The correlation between song elaboration and age in both male and female superb starlings could be similarly shaped by preference for older mates. Several other species have been shown to signal age with song complexity, particularly in other open-ended learners such as willow warblers (*Phylloscopus trochilus*; Gil et al., 2001), swamp sparrows (*Melospiza georgiana*; Ballentine, 2009), and European starlings (*Sturnus vulgaris*; Mountjoy and Lemon, 1995). Superb starlings can live for up to 12 years or more in the wild and breed twice annually (Rubenstein, 2006), meaning that individuals have numerous opportunities to compete for breeding positions during their lifetimes. Therefore, in obligate cooperative breeders like this one, older males could be preferred by immigrant females because they are more likely to have other offspring from previous breeding attempts to act as helpers (Rubenstein, 2006), which are important for increasing the likelihood of successfully fledging offspring (Rubenstein, 2007b). Rather than song complexity increasing with age, an alternative explanation could be that individuals with more complex songs have longer lifespans and are simply over-represented in our study. Additional work will be needed to determine if this is the case, as the data presented here are derived from a cross-sectional sample of the population and do not allow us to test this alternative hypothesis.

Although song diversity appears to increase as starlings become older, those individuals that obtain breeding positions less frequently have more diverse song, a result that contrasts with our initial prediction. This inconsistency may be due to breeders investing more energy in reproduction than in singing or developing more diverse songs. Alternately, breeders may not need to use song displays to compete for breeding positions, as helpers do, since after becoming a breeder, many starlings hold breeding positions for several subsequent seasons (Rubenstein, 2016). Although song diversity may not continue to increase as breeders age, the observed trend of older males and females having more diverse songs suggests that song elaboration is likely to be an honest indicator of social dominance.

Song diversity appears to be shaped by sexual selection in both males and females, but it is also likely to be under strong social selection. Social rank is a key determinant of whether superb starlings will obtain breeding opportunities, and older individuals typically outcompete others for limited breeding positions within social groups (Rubenstein, 2016). Thus, age may be a proxy for dominance status in this species, and a signal of age

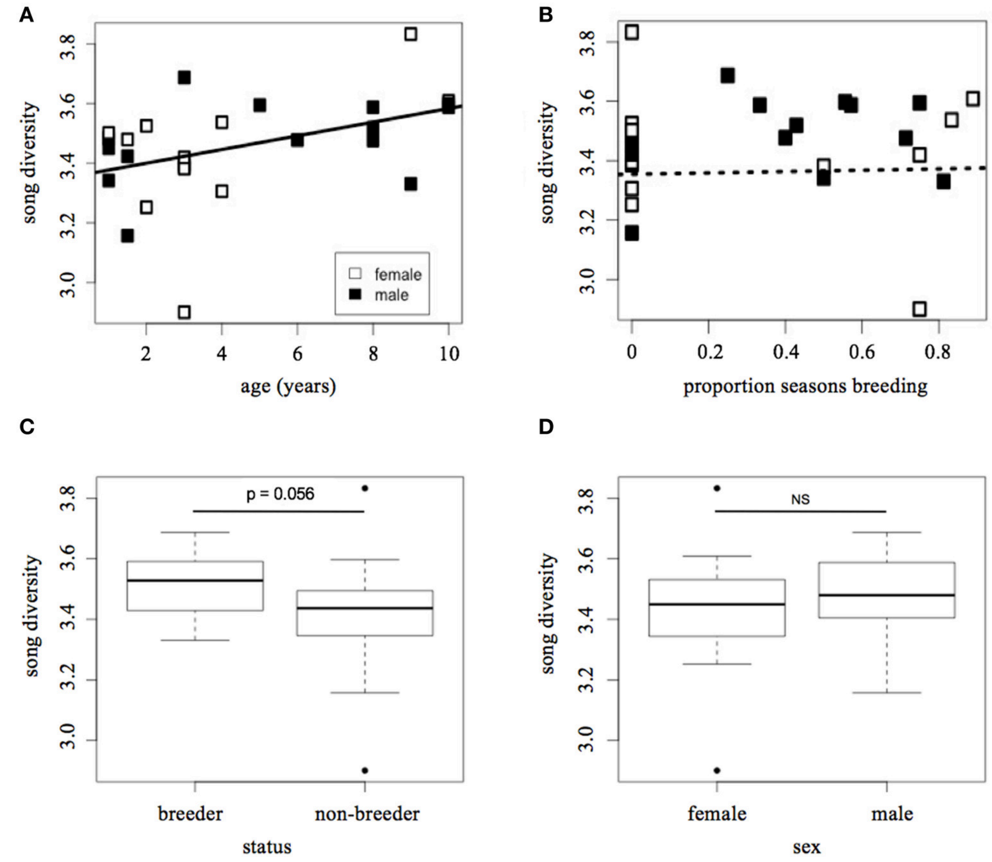

FIGURE 2 | Song diversity vs. indicators of individual fitness and/or social dominance. (A) Song diversity vs. age, **(B)** song diversity vs. proportion of seasons spent breeding, **(C)** song diversity vs. social status, **(D)** song diversity vs. sex. Diversity is calculated using the Shannon Diversity Index (H′). Social status at time of recording (i.e., "breeder" or "non-breeder") was determined from nest observations. Proportion of seasons breeding was measured as the total number of seasons during which an individual held "breeder" status, divided by the total number of seasons in which they were alive and capable of holding a breeding position (i.e., older than 1 year of age). Lines in **(A,B)** represent best fit; the dashed line indicates that the correlation in **(B)** is not statistically significant.

TABLE 1 | Estimate, standard error (SE), degrees of freedom (DF), *t*-values, and *p*-values for each term used in our selected GLMM (in bold print in Table S1).

Variable	Estimate	SE	DF	t	P
Status	0.18	0.087	22.56	2.01	0.056
Proportion seasons breeding	−0.31	0.13	21.96	−2.49	0.021
Sex	0.025	0.062	21.24	0.40	0.69
Age	0.028	0.012	19.82	2.37	0.028

in song could facilitate social competition for shared resources while also helping to establish or maintain rank. The degree to which song diversity is driven by sexual vs. social selection is difficult to tease apart, as dominance status achieved through social competition leads to more breeding opportunities, thereby indirectly influencing fitness. The complexity of the relationship between social and sexual selection, particularly in the context of social rank or dominance status, has been highlighted in recent studies (Lyon and Montgomerie, 2012; Rubenstein, 2012b; Tobias et al., 2012; Clutton-Brock and Huchard, 2013). Although difficult to disentangle, our results are consistent with the hypothesis that song is used in both a sexual and social context in both sexes of superb starlings, as well as with previous work showing that mutual ornamentation can function in signaling dominance and in attracting mates in species with high social competition in both sexes (Kraaijeveld et al., 2004; Viera et al., 2008).

Interestingly, our findings differ from recent work showing that cooperatively breeding striped-headed sparrows (*Peaucaea ruficauda*) show role reversal in song, with females having larger repertoires than males (Illes, 2015), and with evidence of social selection acting more strongly on female song in cooperatively breeding superb fairy-wrens (*Malurus cyaneus*; Cain and Langmore, 2015). This difference may reflect the fact that females of these species experience stronger intrasexual competition for resources than males due to subtle differences in their social systems: striped-headed sparrows live in social groups composed mostly of males (Illes, 2015), and female fairy wrens typically disperse and join shared territories that they often help to defend during males' frequent absences (Cooney and Cockburn, 1995). In contrast, intrasexual competition in superb starlings does not appear to be stronger in females than in males,

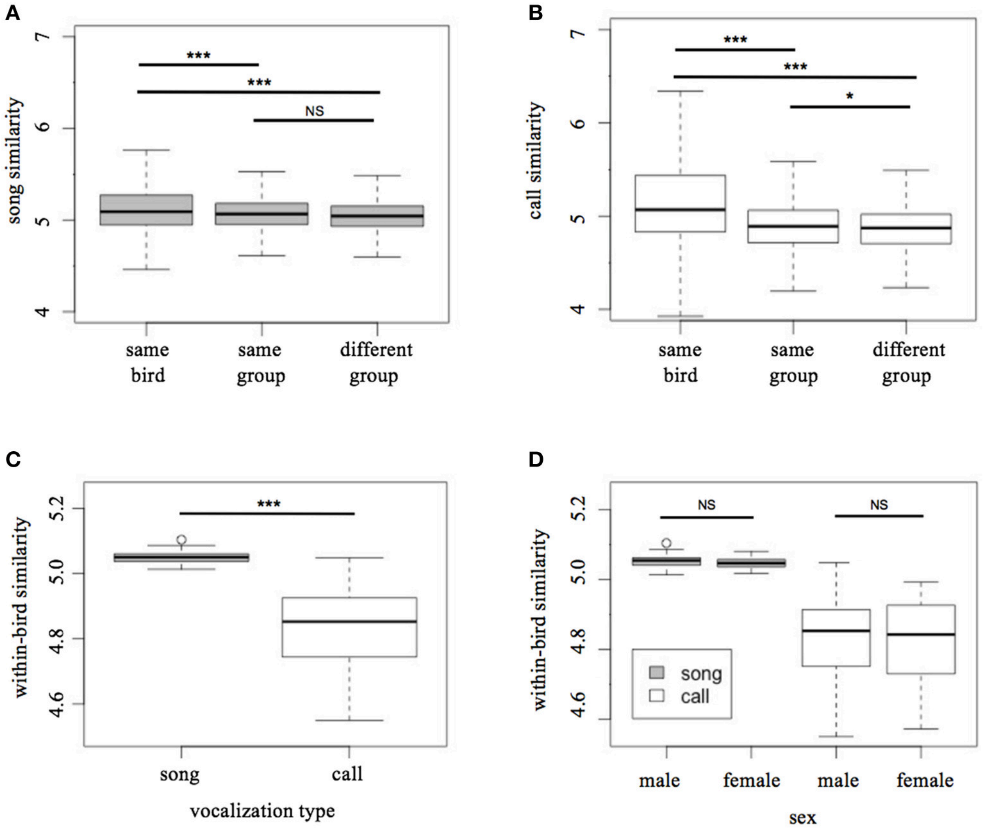

FIGURE 3 | Patterns of similarity in songs and calls. (A) Song motif similarity vs. social relationship, **(B)** call motif similarity vs. social relationship, **(C)** within-bird motif similarity of songs and calls, **(D)** within-bird motif similarity of songs and calls with sexes included. Units for the y-axis are arbitrary. $^{*}p < 0.5$, $^{**}p < 0.01$, $^{***}p < 0.001$.

perhaps resulting from equal sex ratios in social groups or other differences in group composition. Rather, our results support the idea that, despite higher variance in reproductive success among females in cooperative species, female-biased sexual dimorphism (i.e., role-reversal) is usually absent in cooperatively breeding species (Young and Bennett, 2013).

The high levels of within-bird similarity in songs and calls suggest that both types of vocalizations carry a strong signal of individual identity, and may therefore provide a basis for recognition. Although we have shown previously that calls appear to play a role in both individual and group recognition (Keen et al., 2013), the signal of identity in song may serve different purposes due to the specific behavioral contexts in which song is used. Flight calls are often made when entering and leaving a nest or entering a group territory, likely to identify the signaler as a specific individual or group member to prevent agonistic interactions or to encourage cooperation. In contrast, song is produced when perching in the group territory both while alone and in groups (Pilowsky and Rubenstein, 2013). In addition to helping to attract or compete for mates, song displays may be used in establishing social rank, as signaling identity may help to maintain within-sex dominance structures, since such hierarchies are only possible if identities are known (Barnard and Burk, 1979). Furthermore, signals of identity are expected to evolve in systems where individuals have repeated competitive interactions (reviewed in Tibbetts and Dale, 2007), as is the case in superb starling social groups. Because signaling identity while singing may aid in social competition, we hypothesize that this aspect of song is shaped by social selection and could help mediate the competition for social rank in both sexes.

Unlike the pattern observed in flight calls (Keen et al., 2013), song motifs from individuals in the same social group were not significantly more similar to one another than to song motifs from individuals in different groups. In other words, songs do not carry a detectable signal of group identity. This likely indicates that calls play a greater role in group recognition and maintaining boundaries between territories, and that calls may primarily serve to facilitate cooperation within social groups. Future studies should examine the social context in which these signals are produced, as this may offer further insight into the function of both signals.

Taken together, our results show that superb starling song is a complex signal that conveys information about identity and dominance rank. Although playback experiments are needed to demonstrate how this information is used, the present results support the idea that song is under both social and sexual selection to simultaneously express multiple traits (Gil and Gahr, 2002). Additionally, we show that the strength of selection on song diversity and the degree of individuality in songs is similar in males and females, suggesting similar strengths of selection. This may be due to mutual mate choice and high levels of intrasexual competition in both sexes, which is closely linked to the structure of superb starlings' complex social system. Thus, song is likely to be used both in mate attraction and in dominance interactions that influence social rank; these two functions are linked, as dominant individuals are more likely to become breeders (Rubenstein, 2016). Our findings add to a growing body of work suggesting that song can function in both sexual and social contexts, and that the same selective forces can drive trait elaboration in both sexes in cooperatively breeding species.

AUTHOR CONTRIBUTIONS

SK, CM, and DR designed the study. SK, JP collected the data. SK, CM analyzed the data. SK drafted the manuscript. CM, DR revised the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: http://journal.frontiersin.org/article/10.3389/fevo.2016.00046

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HOW DO FEMALES LEARN THEIR SONGS?

Female superb lyrebirds learn vocalisations of other species, with a vocal repertoire that includes mimicry.
Photo: Justin Wellbergen. Audio: Anastasia Dalziell

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Understanding Sex Differences in Form and Function of Bird Song: The Importance of Studying Song Learning Processes

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Birdsong is a culturally transmitted mating signal. Due to historical and geographical biases, song (learning) has been predominantly studied in the temperate zones, where female song is rare. Consequently, mechanisms and function of song learning have been almost exclusively studied in male birds and under the premise that inter- and intra-sexual selection favored larger repertoires and complex songs in males. However, female song is not rare outside the temperate zones and song in both sexes probably is the ancestral state in songbirds. Some song dimorphisms seen today might therefore be manifestations of secondary losses of female song. What selection pressures have favored such losses and other sexual dimorphisms in song? Combined mapping of phylogenetic and ecological correlates of sex differences in song structure and function might provide important clues to the evolution of male and female song. This requires parameterization of the degree of sexual dimorphism. Simple comparison of male-female song might not provide enough resolution, because the same magnitude of difference (e.g., repertoire overlap) could result from different processes: the sexes could differ in how well they learn ("copying fidelity") or from whom they learn ("model selection"). Different learning mechanisms might provide important pointers toward different selection pressures. Investigating sex-specific learning could therefore help to identify the social and ecological selection pressures contributing to sex differences in adult song. The study of female song learning in particular could be crucial to our understanding of (i) song function in males and females and (ii) the evolution of sex-specific song.

Keywords: vocal learning, females, oscines, signal evolution, cultural transmission, signal, plasticity

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BOTH SEXES OF SONGBIRDS SING AND LEARN THEIR SONGS

What is in a name? Song in songbirds (*oscines*) is so ubiquitous and conspicuous that the songbirds, the most speciouse avian clade (comprising almost half of the ~10,000 extant species), were named after it. Song is typically learned early in life from conspecifics—what and how well young birds learn greatly affects the efficacy of their signals as adults (Catchpole and Slater, 2008; Lachlan et al., 2014; Peters et al., 2014). Song is currently the best-studied and probably most widely accepted animal example of a culturally transmitted mating signal (Slater and Ince, 1979; Mundinger, 1982; Podos and Warren, 2007; Riebel et al., 2015).

In most species song functions both as an armament and as an ornament, serving as a keep-away signal to same-sex competitors in the context of resource defense and as a signal to attract and stimulate mates for breeding (Catchpole and Slater, 2008). These functions tally with the canonical male sex role (Andersson, 1994): males can gain fitness by increased investment into sexual signaling to maximize the number of potential partners and exclude competitors. Male song indeed fulfils both functions (Kroodsma and Byers, 1991).

Social learning (typically from conspecifics) is crucial for the development of fully functional song. Ever since Thorpe's seminal studies on chaffinch song learning in the 1950s kick-started modern birdsong research by introducing spectrographic analyses (Slater, 2003; Riebel et al., 2015), the study of the function of birdsong and vocal learning have gone hand-in-hand. However, this inadvertedly became a tale of male song learning only (Riebel, 2003; Riebel et al., 2005): due to historical and geographic research biases, songbirds were studied for many years predominantly in the Passerida of the temperate zones of Europe and North America, where female song is rare (Morton, 1996; Riebel et al., 2005; Odom et al., 2014). This led to the description of birdsong as a predominantly male trait, despite earlier reports of abundant female song in other biogeographic regions (Robinson, 1949; Morton, 1996). Only since the late 1990's has the mounting evidence of female song in other regions and clades resulted in a revision of this view: female song is now understood to be phylogenetically and geographically widespread (Robinson, 1949; Morton, 1996; Langmore, 1998; Riebel, 2003; Hall, 2004; Slater and Mann, 2004; Riebel et al., 2005; Garamszegi et al., 2007; Price, 2015). A recent phylogenetic analysis and ancestral state reconstruction even indicates song in both sexes as the most probable ancestral state (Odom et al., 2014). Current sex differences are thus likely the outcome of both secondary trait loss and selection pressures on sexually dimorphic song (Kraaijeveld, 2014; Odom et al., 2014; Price, 2015). This raises the question of why females stopped singing in some clades but not in others (Odom et al., 2014) and what selection pressures have led to varying degrees of sexual dimorphism (Price, 2015). One promising approach to tackle these questions that has already proven informative for some clades, is to map sex differences in song structure and function and their ecological correlates onto phylogenetic trees to identify common patterns of diversification and losses (Price, 2009; Odom et al., 2015). However, bird song is a mating signal with a twist: substantial phenotypic variation in this trait can arise from cultural transmission and the underlying social learning networks (Lachlan and Slater, 1999). This means that patterns of sex differences can be misleading if the underlying processes causing them are ignored. In the subsequent sections I shall first briefly highlight what we know about the relationships between song learning—both production learning by males and perception learning by females—and the functions of male song. From there I will move on to the question of how studying song production learning in females might provide important cues for hypothesis development regarding the function and evolution of sex differences in male and female song.

PRODUCTION AND PERCEPTION LEARNING AND THE MATE ATTRACTION FUNCTION OF SONG

The mate attraction function of song is well supported by a large body of observational and experimental data from lab and field (Kroodsma and Byers, 1991; Andersson, 1994; Searcy and Yasukawa, 1996; Catchpole and Slater, 2008). There is now increasing evidence that female preferences, like male repertoires, are influenced by cultural transmission (Riebel, 2003). For the few species studied experimentally in this respect, the types of songs females experienced when young are generally preferred over unfamiliar songs in adulthood (Riebel, 2003). Learned preferences thus influence which songs within a population are attractive. This influence is not trivial, but guides mate choice (Riebel, 2003, 2009). In extremis, this can lead to preferences for song of another population within just one generation (Freeberg, 1996, 1998) or to preferences for the song of another subspecies (Clayton, 1990) or a preference for males that mimic the song of new host species in brood parasites (Payne et al., 2000). Song preferences affect mating patterns and gene recombination in the next generation, and for this reason learned mating preferences (for learned traits) are no longer seen as non-heritable phenotypic variation but to affect evolutionary dynamics in time and space (Verzijden et al., 2012). This is particularly true for birdsong, in which gene-culture co-evolution processes are driven by behavioral selection for learning the right types of song well (Lachlan and Feldman, 2003; Lachlan et al., 2013, 2014).

But from whom do females learn? Active song model choice has not been systematically studied in either sex (but for promising methods to approach these questions in wild birds see e.g., Lachlan and Slater, 2003; Templeton et al., 2010; Akcay et al., 2014). Experimental data from song tutoring studies in females show that memorization of preferred songs does not merely reflect availability or exposure frequency: female cowbirds, *Moluthrus ater*, that were raised with controlled exposure to songs preferred as adults those songs that during tutoring had been followed by adult females' "chatter" vocalizations (Freed-Brown and White, 2009). Group-housed young female zebra finches can develop song preferences for their male peers rather than adult tutors (Honarmand et al., 2015). How adult females react to specific variants of male song is thus dependent on their early song experiences (Riebel, 2003), and juvenile social and physical conditions (Holveck and Riebel, 2010; Riebel et al., 2010).

It is likely that in species where males and females sing, similar processes also affect the development of male song preferences. Despite increasing documentation of the potential mate attraction function of female song (Langmore, 1998; Hall, 2004), to the best of my knowledge male song preferences and song based male choice have not been studied, despite empirical evidence for a mate attraction function of female song (Langmore et al., 1996). Males, like females, might also be hormonally stimulated either directly by their partner's or even their own song (Kroodsma, 1976; Cheng, 2003). Interestingly, song can be positively reinforcing even in species with non-singing females, such as zebra finches. Male zebra finches will work for song exposure in operant tasks and prefer to listen to songs of early tutors over unfamiliar songs (Riebel et al., 2002). If mechanisms that combine song memories and behavioral expression of preferences are in place even in species with non-singing females, searching for song preference learning in males might be well worth the while. Some of the methods used for female preference testing, such as phonotaxis paradigms, are likely to work in males as well, because males have been shown to be attracted to and approach playback of female song in species with singing females (Langmore et al., 1996).

PRODUCTION LEARNING AND THE RESOURCE DEFENCE FUNCTION OF SONG

The importance of song in the acquisition and defense of resources (territories, mates, nest sites) is undisputed for male song and male-female duets, and this might also be an important function of female solo song (Cain et al., 2015). But how important is it in this context to sing proper song? Song of male songbirds reared without adult models generally shows impoverished structure but nonetheless contains some species-specific signatures (Marler and Sherman, 1983). Such "isolate" song almost always functions less well (or not at all) in both inter-and intra-sexual contexts (Searcy et al., 1985). However, learning just any species-specific song might not suffice either: In species with clear regional variation, local songs generally elicit stronger territorial responses in playback paradigms where song is used to simulate an intruder (Podos and Warren, 2007; Catchpole and Slater, 2008). Even when learning from the local models only, learning these songs well can be of importance. In swamp sparrows, *Melospizia georgiana,* song variants that match the most typical regional variants in fine detail best, elicit stronger territorial reactions from territory owners—learning precision thus affects same-sex competition (Lachlan et al., 2014). Similarly, learning repertoires of many different songs might improve both a male's resource defense and mate attraction potential (Searcy, 1992; Beecher and Brenowitz, 2005). For male song, what is learned, from whom and how well can thus affect song function.

THE STUDY OF LEARNING MECHANISMS CAN HELP ELUCIDATE FUNCTION AND EVOLUTION OF FEMALE SONG

If song learning affects the efficacy of male songs in mate attraction and resource defense, this could hold for female song too where it fulfils these functions (examples in Langmore, 1998). But if learning song well is so important, why are there such pronounced sex differences in song (learning)? To date, we have no general explanation for the large interspecific variation in sex differences in song quantity and quality which spans the whole range from species with females that never sing (e.g., the zebra finch, Riebel, 2009) to species where females sing more often and more complex songs than males do (e.g., banded wrens *Thryothorus pleurostictus*, Illes and Yunes-Jimenez, 2009). Moreover, sex differences in song go far beyond what might be needed to aid sex recognition (which can also be achieved with simple calls, see e.g., Mouterde et al., 2014; Kipper et al., 2015). Identical functions of song and sexual differentiation of song solely for sex recognition therefore seems a poor and unlikely general explanation for the vast differences in quantity, quality and context that can be found between male and female song (Langmore, 1998; Hall, 2004). It is here where the study of song learning mechanisms might provide important clues to understand the function and evolution of female song.

Identifying when and from whom females learn and whom they try to match e.g., whether they learn pre- or postdispersal, from kin from their natal area or from neighbors when establishing territories, from same- or opposite sex individuals, or their future mates provides important clues as to who might be the most important receivers of these songs. This in turn can help to develop testable hypothesis regarding the function of song. For example if song learning takes place only after dispersal and then only from territory neighbors then being able to song type match neighbors during territory defense is likely of (testable) higher relevance than for example kin recognition (in which case song learning should have taken place pre-dispersal and from relatives).

Knowing how females learn their songs should also enable the construction of more informative phylogenies. If song sex differences are scored solely by defining "maleness" of female song by looking at percentage shared song elements, ignoring learning, then we will obtain different trait values than when comparing repertoire size or learning accuracy. The hypothetical examples in **Table 1** are intended to illustrate this point: the first column shows schematic spectrograms representing a male and female song in a hypothetical songbird species. The two types of song are roughly of the same length and comparable complexity (both songs contain a 2-note syllable, a whistle note and a buzz note). The second and third column illustrate how male and female song is expected to look in the next generation under each of two different scenarios: (I) sex-specific model choice where males copy selectively from males and females selectively copy from females and (II) sex-specific copying fidelity where both sexes only partially copy their chosen models (some elements are missing, and there is some blending of the different model song types) but overall, one sex (here the male, in line with the classic view) imitates more components and does so more accurately. Below these song examples, I listed four parameters that are often used to score song sex differences. Notably, the two scores that take learning processes into account yield different patterns of scored sex differences than the two scores comparing males and females while ignoring model choice and copying fidelity. Analyses taking song learning into account would score no sex differences in amount and ability of learning under the sex-specific learning strategies in scenario I, but register a sex difference for the songs in scenario II. In contrast, analyses scoring sex differences by looking only at male-female repertoire sharing would arrive at the opposite conclusion: a maximum sex difference score under sex-specific model choice in scenario I and a less pronounced sex difference in scenario II (sex-specific copying fidelity). Song parameter choice thus can affect both the direction and magnitude of sex differences.

This is of consequence for our attempts at constructing phylogenies: Evolutionary patterns often can only be discovered when traits are scored continuously rather than dichotomously (Dale et al., 2015; Price, 2015). The above examples illustrate that trait values can yield different results with regard to sex differences in song depending on whether learning processes are ignored or included. It is perhaps too early to speculate which of these measures is the most informative. For now, I hope to raise awareness for (a) that systematically scoring song differences between sexes with one method for all species must be premium for comparative studies and (b) that unraveling the song learning mechanisms and social model choice in combination with study of the interactions between sex-specific contexts and contents of song might provide important hints as to the function of evolution of these sex differences.

NOT ALL SEX DIFFERENCES IN SONG ARE INDICATIVE OF SEX DIFFERENCES IN SONG LEARNING CAPACITY

A final note of caution: sex differences can also be caused by other than social learning processes and not all sex differences in adult song necessarily reflect different learning strategies. Aspects of male and female physiology could differ such that even when both sexes learn the same songs (equally well) their songs sound different (Yamaguchi, 1998) because (1) sex differences in vocal tract anatomy affect vocal output (Ballintijn and ten Cate, 1997) and (2) seasonal and/or sex specific androgen levels could cause sex differences if females do not fully crystallize their song due to lower androgen levels. However, these questions as yet lack systematic study in songbirds (Gahr, 2014) and there are also observations of males and females that show no pronounced sex differences in song despite different steroid levels (Schwabl et al., 2015).

Moreover, physiological mechanisms and learning strategies can interact in multiple ways. And no learning strategy fits all: sex differences could come about because the sexes differ in different aspects of their learning strategies e.g., (1) one sex learns more or more accurately than the other (see **Table 1**), because (2) there are sex differences in the timing of the sensitive phase (Nelson et al., 1997; Yamaguchi, 1998) or (3) as a side effect of different habitat usage and/or dispersal patterns males and females are exposed to different models or (4) show differences in active model choice, meaning that they either pick different social models (see **Table 1**) or pick different song models from different tutors (Geberzahn and Gahr, 2013). The most conspicuous variant of the latter strategy would be true sex-specific lineages, where both sexes have specific vocalizations and learning takes place between same-sex individuals only (Price, 1998). This has been

TABLE 1 | Scoring song sex differences with and without taking learning processes into account yields different results.

		TUTEES **Sexes could differ in**	
	MODELS	**I Model choice**	**II Copying fidelity**
♂	A 		
♀	B 		
		↓	↓
		Measured sex differences	
SONG LEARNING			
% notes of tutee's song copied from tutor(s)		M = F	M > F
Total # of learned notes		M = F	M > F
SONG STRUCTURE (IGNORING LEARNING)			
Complexity (e.g., # different notes/total # notes)		M = F	F > M
"Maleness of song" (e.g., notes shared F/M)		0/4	2/5

Top panel: A and B represent a male (black) and female (orange) model song in a hypothetical songbird species. The songs are of comparable complexity: each has one 2-note syllable, one whistle note and one buzz note. With I) sex-specific model choice, male and female tutees end up with highly divergent songs (low "maleness" of female song) although both accurately learned from their respective model. In II) there is no sex-specific model choice and both sexes learned from more than one model but the male and female differ in how much they learned and how well ("copying fidelity"). In the example in the 3rd column, all notes in the male's song are accurate copies. In the female's song, comparison with the tutors' songs shows that not all notes are accurate copies and that there is also an improvised note type (in gray). Lower panel: This table illustrates how scored sex differences might differ depending on whether information on learning processes such as model choice or copying fidelity are included or not. For example, comparing each male and female with their respective model, yields no sex differences in amount and ability of learning in scenario I (Male M = Female F) but differences between males and females in how well they copied in scenario II (M > F). In contrast, an analysis scoring male-female note repertoire sharing, would arrive at the opposite conclusion: a maximum sex difference score in scenario I and less pronounced sex differences in scenario II. Song parameter choice thus can affect both the direction and magnitude of measured sex differences.

hypothesized for a number of species, but there has not yet been systematic study in a single species that was able to exclude all possible alternative explanations (for review and discussion see Riebel, 2003).

CONCLUSIONS AND OUTLOOK

To summarize and conclude: female songbirds in many species sing and learn which songs to sing. Females of both singing and non-singing species have been documented to also acquire their song preferences through social learning processes (Riebel, 2003). Learning and cultural transmission processes deeply impact the efficacy of the signal and both learned song and preferences are subjected to natural, social, inter- and intra-sexual selection processes. The timing of sensitive phases and mechanisms of model choice but also learning-unrelated behavior such as (sex-specific) dispersal patterns all impact song and eventually fitness. Ideally, the study of how developmental processes contribute to inter-individual variation in traits and preferences should go hand in hand with studies of song function. Questions we might want to ask in future studies investigating sex differences in song (and their costs and benefits) include:

1. Do males and females differ in the timing of song learning?
2. Are there sex differences in model choice (who is learning from whom)?
3. What is learned and how accurately, and does this depend on sex?
4. Is there evidence for sex-specific habitat usage and/or dispersal patterns that will lead to different model availability in males and females?

Identifying sex-specific learning strategies might provide important clues to the selection pressures on sexual differentiation of song. Asking why females in one species needn't learn precisely while in another exact copying (and from selected models) is important might lead us to the social factors selecting for particular learning strategies. There has been no systematic study of these questions in females yet, but systematic comparisons of how learning contributes to inter-individual variation in signaling and signal decoding will provide important steps toward unraveling the function(s) of intra-and inter-sexual song variation.

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and approved it for publication.

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Superb Fairy-Wren (*Malurus cyaneus*) Sons and Daughters Acquire Song Elements of Mothers and Social Fathers

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Birdsong is regarded as a classic example of a sexually-selected trait and has been primarily studied in systems with male song. Complex solo female song is emerging from the shadows of overlooked phenomena. In males, rearing conditions affect male song complexity, and males with complex songs are often more successful at mate attraction and territorial defense. Little is known about the ontogeny or function of complex female song. Here we examine song elements in fledgling superb fairy-wrens (*Malurus cyaneus*) in relation to the song elements of adult tutors. Male and female superb fairy-wrens produce solo song year-round to defend a territory. We ask if sons and daughters acquire song elements from sex-specific vocal tutors. We found that sons and daughters produced the song elements of their mothers and social fathers, and that sons and daughters had comparable song element repertoires at age 7–10 weeks. We conclude that sons and daughters increase their song element repertoire when vocally imitating elements from several vocal tutors, and that both sexes acquire elements from male and female vocal tutors in this system.

Keywords: superb fairy-wren, birdsong, female song, vocal learning, song element repertoire, Maluridae, vocal tutors

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INTRODUCTION

Birdsong is widely regarded as a sexually-selected trait given ample evidence that complex male song is more likely to attract females and repel intruder males (Andersson, 1994; Marler and Slabbekoorn, 2004; Catchpole and Slater, 2008). There is growing evidence that female song is "common" rather than "exceptional" (Riebel et al., 2005; Garamszegi et al., 2007; Price, 2009; Odom et al., 2014), which focuses research attention on the role of sexual selection for complex female song (Price, 2015). Song complexity in females varies greatly across species (Odom et al., 2014; Price, 2015). As discussed by Price (2015): "Although female song is often treated as a discrete binary character in comparisons among species, with some species categorized as having female song and others not (e.g., Garamszegi et al., 2007; Price, 2009; Odom et al., 2014), evidence suggests that female singing can vary continuously across taxa in both expression and complexity." To date, we know very little about how this complexity in female song arises.

Female song functions for multiple purposes, including territorial defense, mate attraction, coordination of breeding activities, and female-female competition for reproductive resources (Langmore, 1998). In the superb fairy-wren (*Malurus cyaneus*), female song, similar to male song, has been shown to function in response to the threat of same-sex conspecific intruders in the territory (Kleindorfer et al., 2013; Cain and Langmore, 2015). In banded wrens (*Thryophilus pleurostictus*), there is support for the function of female song for communicating with their breeding partner (Hall et al., 2015), whereas the main functions of male song are mate attraction and stimulation, and territorial advertisement and defense (Kroodsma, 1976; Catchpole and Slater, 2008). Therefore, the evolution of male and female song could be under the same or different selection pressures depending on the specific context (Price, 2015).

Song can be a sexually dimorphic trait. Generally, males sing more complex songs than females (discussed in Brenowitz and Kroodsma, 1996; Catchpole and Slater, 2008). Some studies have found comparable song structure, complexity, and repertoire size in males and females (Brunton and Li, 2006; Pilowsky and Rubenstein, 2013; Schwabl et al., 2015). While females rarely have larger song repertoires than males, there are exceptions: female stripe-headed sparrows (*Peucaea r. ruficauda*) have been shown to have more complex chatter song repertoires than males (Illes, 2015), and female Australian magpies (*Gymnorhina tibicen*) had larger duet repertoires than males (Brown and Farabaugh, 1991). The capacity to learn complex songs is strongly influenced by the development of the neural song system (Buchanan et al., 2004). In general, male songbirds have larger high vocal center (HVC) nuclei than females (MacDougall-Shackleton and Ball, 1999; Hall et al., 2010), yet female song can be similar or more complex than male song (Illes, 2015; Schwabl et al., 2015). This suggests that a smaller HVC in female songbirds is not necessarily a constraint for learning and developing complex songs.

There is limited knowledge of song learning in females, including how females learn to sing and from whom (Riebel, 2003; Riebel et al., 2005). In species with male and female song, whereby the song may be the same or different across the sexes, both sexes are potential song tutors. When both parents sing, young birds could learn their song from both parents or from same-sex vocal tutors. Studies on captive birds show that young male and female songbirds learn primarily from same-sex vocal tutors in slate-colored boubous (*Laniarius funebris*; Wickler and Sonnenschein, 1989), stripe-backed wrens (*Campylorhynchus nuchalis*; Price, 1998), and European starlings (*Sturnus vulgaris*; Hausberger et al., 1995). Young birds learn from both male and female song tutors in Indian Hill mynahs (*Gracula religiosa*; Bertram, 1970), Northern cardinals (*Cardinalis cardinalis*; Yamaguchi, 2001) and blue-capped cordon-bleus (*Uraeginthus cyanocephalus*; Geberzahn and Gahr, 2013; Lobato et al., 2015).

Our study species is the superb fairy-wren, a long-lived (up to 11 years) sedentary and territorial songbird (Rowley and Russell, 1997; Dunn and Cockburn, 1999). Both males and females sing solo chatter song year-round (Cooney and Cockburn, 1995; Cain and Langmore, 2015). The chatter song has several proposed functions including territory defense, mate attraction and within-pair communication (Cooney and Cockburn, 1995; Cockburn et al., 2009; Cain and Langmore, 2015). Recent evidence suggests that the primary function of chatter song is intrasexual competition for resources including social mates and territories (Cooney and Cockburn, 1995; Cockburn et al., 2009; Kleindorfer et al., 2013; Cain and Langmore, 2015; Cain et al., 2015). A secondary function of chatter song may be within-pair communication because females sing on the nest in response to their social male's song (Kleindorfer et al., 2016). It remains untested if males and females pair assortatively for song.

We study song element types in the subsong of fledgling superb fairy-wrens in relation to the song element types of the social father and mother, and ask if there are sex-specific vocal tutors. Individual fairy-wrens have different element types per song (Kleindorfer et al., 2013) therefore, we predict within-pair differences in chatter song element types. Within pairs, we predict that the male and female will have "shared" element types produced by both members of the pair and "within-pair unique" (hereafter "unique") element types produced by only the male or female within the pair (but not necessarily unique to the population). We predict that sons and daughters sing the "shared" parent element types because irrespective of vocal tutor type (male or female), the "shared" element types will be present in the vocal repertoire of the tutor(s). We also predict that sons and daughters produce different proportions of "unique" elements, whereby sons produce the "unique" element in the social father's element repertoire and daughters produce the "unique" element in the mother's element repertoire. This prediction rests on the idea that sons and daughters learn element types that signal their gender because birds acoustically discriminate sexes and chatter song functions for intrasexual competition (Cockburn et al., 2009; Kleindorfer et al., 2013; Cain and Langmore, 2015). Finally, we predict that the presence of helper males in natal groups will affect element repertoire size in male and female offspring. If there is sex-specific vocal tutoring, then we predict that the presence of helper males will increase element repertoire size in the subsong of sons but not daughters. If, however, the number of vocal tutors (and not their gender *per se*) increases element repertoire size in both sons and daughters, then we predict that the presence of helper males in natal groups increases element repertoire size in the subsong of sons and daughters.

MATERIALS AND METHODS

Study Sites

This study on song element acquisition in the superb fairy-wren was carried out at two field sites (1) Cleland Wildlife Park (34°58′S, 138°41′E) and (2) Scott Creek Conservation Park (35°05′S, 138°41′E) in the Mount Lofty Ranges, South Australia. We recorded adult chatter song and fledgling subsong from 11 family groups across three field seasons (September–February 2012, 2013, 2014).

Study Species

The superb fairy-wren is an insectivorous passerine found in south-eastern Australia, and is a member of the Maluridae family (Rowley and Russell, 1997). The superb fairy-wren has a cooperative breeding system with a socially monogamous male and female pair, often assisted by one or more subordinate males (helpers) that provision the young and defend the permanent territory (Rowley, 1965; Mulder et al., 1994; Dunn et al., 1995; Rowley and Russell, 1997; Mulder, 1997; Cockburn et al., 2008). The dominant male is not always the genetic father because most broods (75–95%) contain young sired by extra-pair males (Mulder et al., 1994; Cockburn et al., 2003; Colombelli-Négrel et al., 2009). We refer to the dominant male as the social father. The breeding season occurs between August and February, with 1–3 breeding attempts per year and 2–3 eggs per nest (Colombelli-Négrel and Kleindorfer, 2009). The incubation phase has a duration of 12–15 days and the nestling phase is 10–15 days (Colombelli-Négrel and Kleindorfer, 2009). Nest predation is high (e.g., 24–74%; discussed in Rowley and Russell, 1997; Colombelli-Négrel and Kleindorfer, 2009) and once fledged, there is the risk of fledgling predation (Rowley, 1965; Cockburn et al., 2008). Most groups produce one brood of fledglings per year (Rowley and Russell, 1997). Females are uniparental incubators; all group members feed nestlings and dependent fledglings (Mulder et al., 1994; Dunn et al., 1995). Fledglings become independent of adult feeding around 4 weeks after fledging, but remain in the natal group for several months (Mulder, 1995; Rowley and Russell, 1997). Males are philopatric, remaining in the natal territory for one or more years as helper males, whereas females disperse in the first year, on average 1–10 km and 11.8 territories removed from the natal territory (Rowley, 1965; Cooney and Cockburn, 1995; Mulder, 1995; Cockburn et al., 2003; Double et al., 2005). Adult males and females are sexually dichromatic and can be easily distinguished in the field. Recently fledged young of both sexes resemble adult females (Mulder, 1995).

There are eight vocalizations described for the superb fairy-wren (Rowley, 1965; Kleindorfer et al., 2013). We focus on chatter song (Type I song) because it is the most common song produced year-round by both male and female superb fairy-wrens for territorial and resource defense (Langmore and Mulder, 1992; Cooney and Cockburn, 1995; Kleindorfer et al., 2013; Cain and Langmore, 2015). The chatter song is a variable, complex song that consists of ~8 structurally distinct element types produced ~50 times per song for a duration of ~3 s (Langmore and Mulder, 1992; Kleindorfer et al., 2013). Fledglings begin singing subsong from 4 weeks after fledging (Rowley, 1965; Langmore and Mulder, 1992). It is not known when fledgling subsong crystallizes, but 1-year old birds sing full adult song (Rowley, 1965). Adult males sing longer, more complex songs than females in some populations (Kleindorfer et al., 2013). Males also have a larger song repertoire than females: males sing chatter song and trill song (Type II song) to attract extra-pair copulations (Langmore and Mulder, 1992; Cooney and Cockburn, 1995; Dalziell and Cockburn, 2008; Cockburn et al., 2009; Colombelli-Négrel et al., 2011). There is evidence that male trill song is learned: introductory elements of trill song were more similar between males and their social fathers than males and their genetic fathers; males that dispersed from the natal territory acquired the local trill song dialect (Blackmore, 2002). Furthermore, sons have been shown to learn Type II song (similar to superb fairy-wren trill song) from their social fathers in the splendid fairy-wren (*M. splendens*), a closely-related *Malurus* species (Greig et al., 2012).

Nest Monitoring

We monitored a total of 125 superb fairy-wren nests over 3 years. Nests were monitored every 2–4 days to check the status of the nest (building, eggs, nestlings, fledged) and nesting outcome (eggs depredated, eggs abandoned, nestlings depredated, nestlings abandoned, nestlings fledged). Of these 125 nests, 82 were depredated (65%), and 19 nests produced fledglings (15%). In this study, we analyzed song recordings for 11 nests (49 birds) for which we have recordings of both parental chatter song (male and female; $N = 22$ birds), helper males ($N = 9$) as well as all offspring ($N = 17$). For each nest, we had a minimum of three song recordings per individual bird (dominant male, dominant female, helper male, fledged young).

Of the 31 adult birds for which we have song recordings, we color banded and measured 18 birds using target mist-netting. We banded at least one dominant male or female at 10 nests and six helper males at the five nests with helper males. For individuals that were not banded, we were certain of their identity based on group size and composition and the interactions of unbanded birds with banded birds (e.g., group foraging together, feeding fledglings). Nine out of 11 nests contained a single unbanded bird; one nest had an unbanded dominant male and female; one nest had an unbanded dominant female and helper male. Each captured individual was marked with a unique combination of plastic color bands and a numbered aluminum band provided by the Australian Bird and Bat Banding Scheme (ABBBS). Nestlings were banded 7–8 days after hatching.

We sexed the fledglings using the standard avian sexing method outlined by Griffiths et al. (1998), using primers P8 (5′-CTC- CCAAGGATGAGRAAYTG-3′) and P2 (5′-TCTGCATC-GCTAAATCCTTT-3′) with modifications to the protocol as follows. We carried out PCR amplification in a total volume of 24 μl with PCR reagents in following final concentrations: 1 XμM MRT buffer, 0.2 μM of each primer, 0.5 units Immolase and between 10 and 100 ng DNA. PCR conditions were an initial denaturing step at 94°C for 10 min, followed by 35 cycles of 94°C for 45 s, 48°C for 45 s, and 72°C for 45 s. The program was completed with a final run of 72°C for 5 min and 25°C for 2 min. Of the 17 fledglings, eight were male and nine were female.

The research was approved by the Animal Welfare Committee of Flinders University (permit number E386), which operates under the *Animal Welfare Act 1985* (SA). Permit to undertake scientific research in SA was granted by the SA Department of Environment, Water and Natural Resources (permit number Z24699-11). All birds were banded under permit (banding authority number 2601) from the Australian Bird and Bat Banding Scheme.

Song Recordings

We recorded the chatter song of 11 dominant pair males, 11 dominant pair females, nine helper males, eight sons, and nine daughters, from 11 nests. We recorded adult birds opportunistically throughout the breeding season. We recorded the song of fledgling birds at a distance of 5–10 m from the bird every 14 days for 8 weeks post-fledging. Recordings were made with a Telinga Twin Science parabolic microphone (Telinga Microphones, Sweden) connected to a portable Sound Device 722 digital audio recorder (Sound Devices, USA). Sound files were recorded as broadcast wave files (24 bit, 48 kHz).

Acoustical Analyses

We transcribed all sound files to an Apple Macpro (Apple, USA) for editing with Amadeus Pro 2.1.2 (HairerSoft, Switzerland) and analysis with Raven Pro 1.5 (Charif et al., 2010). Spectrograms were created for 3–5 songs per individual using the Hann algorithm (filter bandwidth 270 Hz, size 256 samples, time grid overlap 50%, grid resolution 2.67 ms, 188 Hz, DTF 256 samples). We scored the number of different element types per song for every bird. We refer to the number of different element types per bird, summed over the 3–5 songs analyzed, as the song element repertoire. In this study, we did not quantify the absolute element repertoire size. Using the available data of 3–5 songs per individual, there was no statistical association between the number of songs analyzed per bird and the element repertoire size (data available upon request). We refer to the combined number of different element types between two or more individuals (for example, the pair) as song element diversity. We define an element in the song as a single, continuous trace on a spectrogram. We created an element library (**Figure 1**) based on the existing element classifications developed by Langmore and Mulder (1992), Blackmore (2002), Dalziell and Cockburn (2008), Colombelli-Négrel et al. (2011) and Kleindorfer et al. (2013), and comparing these classifications of elements to songs of our monitored populations. We identified 10 element types that have previously been classified in different populations (A, F, O, P, Q, R, T, U, V, W,) and 6 new elements found in our populations (FL, G, K, L, Z, ZN). Within pairs, we identified element types that were present in both the male and the female songs; these element types are referred to as "shared" elements. Within pairs, we identified element types that were present in either the male or the female; these element types are referred to as "within-pair unique elements" (hereafter referred to as "unique"; we note that within-pair "unique" elements may occur in other fairy-wren individuals and hence are not "unique" to the individual but just within the pair).

We used spectrogram cross-correlation (SPCC) analysis to examine the similarity between different element types, using five examples from different individuals per element type (Raven Pro 1.5, Cornell Lab of Ornithology; band-pass filtered from 500 to 12000 Hz). SPCC produces a matrix of similarity (S), which we transformed into a matrix of distance (D) using the transformation [D = (1–S) 0.5]. This matrix was evaluated by principal coordination analysis (PCoA) using the R package for multivariate and spatial analysis, version 4.0 (Casgrain and Legendre, 2001), as outlined in Colombelli-Négrel et al. (2012). The PCoA gives several coordinate values per element and the first five coordinate values explain most of the data set, which we used as similarity values (Baker and Logue, 2003). To determine if the assigned element categories were significantly different from one another, we averaged the five PCoA coordinate values into a single measure and analyzed the mean coordinate values per element type in a univariate ANOVA using IBM SPSS 22 (SPSS Inc, Chicago, USA). The 16 different element types were significantly different [$F_{(14,60)} = 1.919$, $P = 0.042$]. We used these element categories to classify the different element types per song, and the song element repertoire per individual (**Figure 1**). We printed spectrograms and assigned element types visually according to the library of element categories. This method was chosen because humans outperform machines for tasks like the visual recognition of element types (Law and Ahn, 2011). Three people (two naïve assessors and the person who scored the spectrograms for this study) independently reviewed 20 randomly chosen spectrograms (identity of bird unknown) and classified the different element types. The average similarity rate was 95.7% for the scoring of the number of different element types.

Statistical Analyses

We used IBM SPSS 22 for statistical analyses of song element types. We examined the data for normality and homogeneity of variance. The variables "different element types per social father" and "different element types per mother" were square-root transformed, and "number of unique paternal elements" and "number of unique maternal elements" were reflect and square-root transformed to satisfy requirements of normality for parametric tests.

RESULTS

Song Element Repertoire of Social Father and Mother Pairs

There were 16 different song element types in the repertoire of the 11 males and 11 females that were social parents (**Figure 1**). No element was exclusive to males or females in the study population, and hence there were no "male elements" vs. "female elements." Element repertoire size was statistically comparable between social fathers (8.5 ± 0.6; mean ± se) and mothers (8.5 ± 0.4) for 11 pairs [paired samples *t*-test: $t_{(10)} = 0.064$, $P = 0.950$]. The element diversity (combined element repertoire of social father and mother) between pairs varied (mean: 10.7 ± 0.4, range: 9–14).

Within each pair, 60.2 ± 3.7% of song elements were present in both the social father and mother song, and these elements are referred to as "shared." The percentage of elements present in only the social father or mother song was 39.8 ± 3.7%, and these elements are referred to as "unique." The mean number of "shared" element types within pairs was 6.4 ± 0.3 element types. The number of "unique" elements did not differ significantly between social fathers (2.2 ± 0.6) and mothers [2.2 ± 0.3; paired samples *t*-test: $t_{(11)} = 0.530$, $P = 0.608$; **Figure 2**].

Song Element Repertoire of the Sons and Daughters

Song element repertoires were comparable in sons (9.3 ± 0.8) and daughters [10.2 ± 0.6; independent samples *t*-test per nest: $t_{(12)} = -0.813$, $N = 14$ birds, $P = 0.432$; **Figure 2**]. The average number of different element types per brood varied (mean: 8.9 ± 0.5, range: 6–11.5). There was no significant correlation between the element diversity of the social father and mother, and brood [linear regression: $F_{(1,9)} = 0.119$, $r = 0.114$, $P = 0.738$] or group (parents + helper male) and brood [linear regression: $F_{(1,9)} = 0.028$, $r = 0.955$, $P = 0.871$].

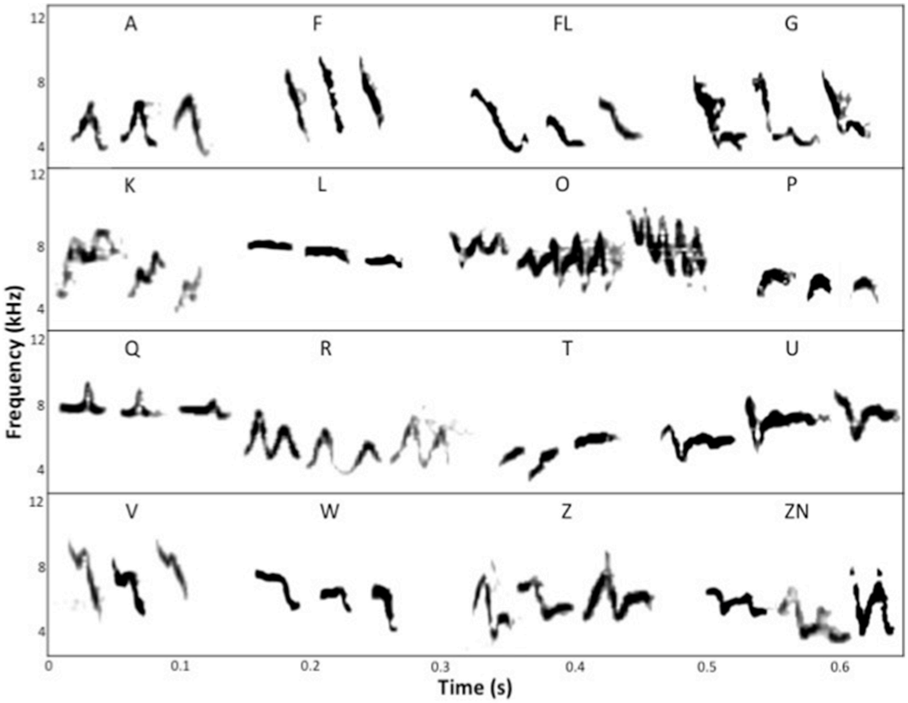

FIGURE 1 | Spectrograms of different element types identified in the chatter song of superb fairy-wrens (*Malurus cyaneus*) from populations in the Mount Lofty Ranges region of South Australia. Each element type is illustrated with three exemplars. The elements identified in these populations include both existing element classifications and novel element types. The existing element types are: A, F, O, P, Q, R, T, U, V, W. The novel element types found in the studied populations are: FL, G, K, L, Z, ZN.

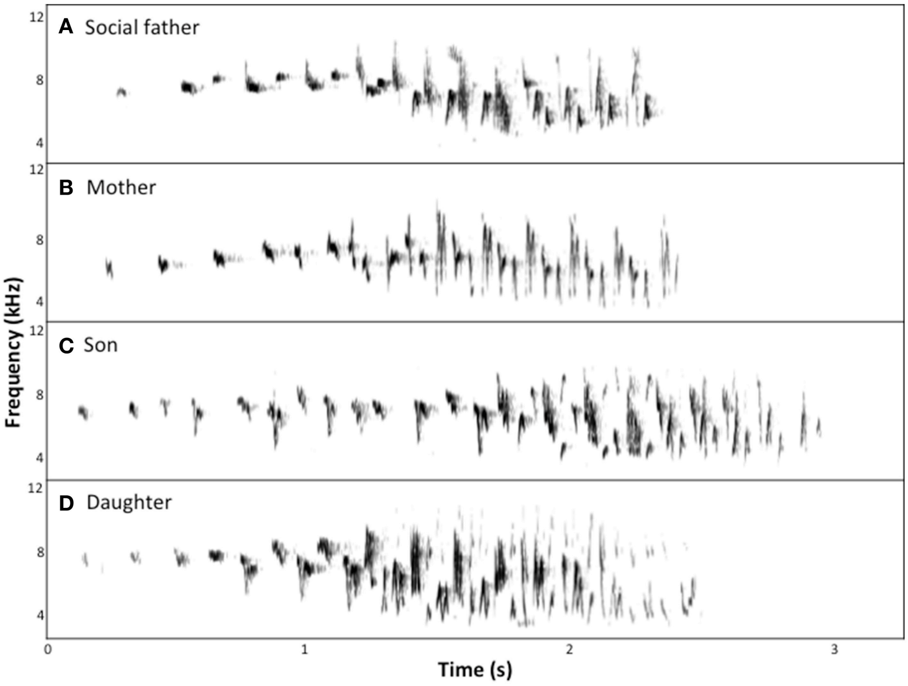

FIGURE 2 | Spectrograms of chatter song of a family group of superb fairy-wrens: (A) social father; (B) mother; (C) son, aged 9 weeks; (D) daughter, aged 9 weeks.

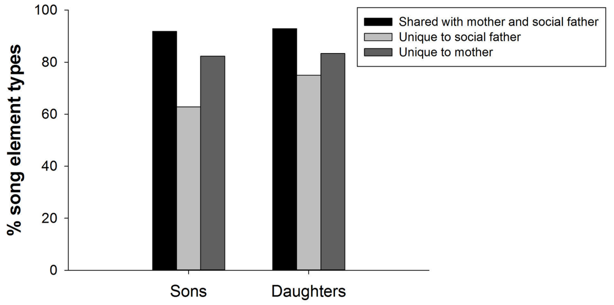

FIGURE 3 | The mean percentage of different song element types in the element repertoires of fledged superb fairy-wren sons ($N = 8$) and daughters ($N = 9$). Bars show the percentage of elements in the songs of sons and daughters that were "shared" with the social father and mother, "unique" to the paternal song, or "unique" to the maternal song.

"Shared" and "Unique" Song Elements Present in Sons and Daughters

The number of "shared" elements from their social parent vocal tutors was comparable in sons (5.6 ± 0.4) and daughters (6.5 ± 0.2; sign test: $P > 0.999$; **Figure 3**). Of the total song elements produced by fledglings, there was no significant difference between the percentage of "unique" maternal elements (86.0 ± 10.3%) and the percentage of "unique" paternal elements (64.3 ± 11.2%; sign test: $P = 0.125$). Fledglings that sang "unique" element types from both the mother and social father produced a higher number of different element types (10.3 ± 0.6) than fledglings that did not sing "unique" element types from both social parents [7.3 ± 0.9; independent samples t-test: $F_{(10)} = 0.417$, $t = -2.915$, $P = 0.015$]. Looking at each sex separately (using the average per sex per nest), sons did not differ significantly in the number of "unique" maternal (1.9 ± 0.2) or paternal (1.3 ± 0.5) elements (sign test: $P = 0.289$), and daughters did not differ significantly in the number of "unique" maternal (1.2 ± 0.3) or paternal (1.5 ± 0.3) elements (sign test: $P > 0.999$; **Figure 3**). Five fledglings had "unique" element types (1.4 ± 0.2) not present in the song repertoire of the social father, mother, or helper male.

Song Element Repertoire of Helper Male in Relation to Dominant Pair Male and Female

Of the 11 nests, five nests had helper males (one nest had one helper male and four nests had two helper males). Among helper males, most element types (56.4% ± 2.8) were "shared" with the dominant pair, some elements were the same as the "unique" maternal element types (15.3% ± 3.9), some elements were the same as the "unique" paternal element types (13.6% ± 3.9), and some were "unique" to the helper male (14.6% ± 3.0). Expressed as a number rather than a percentage, helper males had 1.6 ± 0.4 "unique" element types that were different from the "unique" element types in the pair male and female. The number of different element types produced by all adult birds (pair + helper males) was not significantly different between nests with and without helper males [independent samples t-test: $t_{(9)} = -1.393$, $P = 0.197$].

Song Element Repertoire of Sons and Daughters in Relation to Groups with and Without Helper Males

There was no significant difference between the element repertoire size of fledglings in groups with helper males (9.6 ± 1.0) and without helper males [9.1 ± 0.7; independent t-test: $t_{(10)} = -0.372$, $P = 0.718$]. Examining each sex separately, the element repertoire size of sons in groups with helper males was comparable with the element repertoire size of sons in groups without helper males [independent t-test: $t_{(6)} = -0.551$, $P = 0.601$]; the element repertoire size of daughters in groups with helper males was comparable with the element repertoire size of daughters in groups without helper males (Mann-Whitney U-test: $U = 4.000$, $z = -0.221$, $P = 0.825$; **Figure 4**).

DISCUSSION

The main findings of this study are that (1) the chatter song in nesting pairs of superb fairy-wrens contained "shared" element types found in both male and female chatter song as well as "unique" element types produced by only one parent (male or female); (2) sons and daughters sang "shared" and "unique" song element types of both social father and mother (parental) vocal tutors; (3) sons and daughters had comparable song element repertoires at age 7–10 weeks; (4) the presence of helper males did not increase the element repertoire size of fledglings. These findings suggest that sons and daughters acquire song element types from both male and female tutors. Because we did not assess maximum repertoire size in adult or fledgling birds or examine song element types of neighboring birds, sons and daughters may produce vocal elements from individuals outside their social group.

Within-Pair "Shared" and "Unique" Song Element Types

Males and females within-pairs produced element types that were either present in the chatter song of both parents, or element types present in only one parent. Repertoire composition was variable across individuals, with no consistent sex differences in the presence or absence of particular element types. Other studies have found both sex-specific and individual-specific element types. Male and female bellbirds (*Anthornis melanura*) shared 20% of syllable types but most syllables (80%) were sex-specific at a population level (Brunton and Li, 2006). In comparison, male and female Australian magpies rarely shared syllable types for carol songs, and syllable repertoires were highly individual-specific (Brown and Farabaugh, 1991). Our study was limited to the comparison of shared and unique elements within pairs and not at the population level. At the population level, there were sex differences in the number of particular element types; similar to our study, there were no sex-specific element types (Kleindorfer et al., 2013).

Sons and Daughters Sang Element Types of Mothers and Social Fathers

Our finding that offspring produced the "unique" maternal and "unique" paternal element types supports the idea that

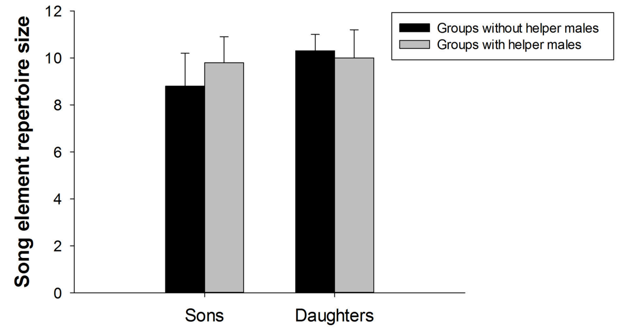

FIGURE 4 | The song element repertoire size (number of different element types; mean ± se) in fledged superb fairy-wren sons (*N* = 8) and daughters (*N* = 9). Bars show the element repertoire size in sons and daughters from groups without helper males (social father, mother and brood) and groups with helper males (social father, mother, helper males, and brood).

superb fairy-wren mothers and social fathers were vocal tutors for sons and daughters. However, we did not compare the parental element repertoire with neighboring individuals, so fledglings could also have learned these element types from individuals other than the social parents. Because we did not cross-foster clutches, we cannot rule out innate components of element repertoire emergence in sons and daughters. It is well-established that oscine passerines learn their songs (Brenowitz, 1991; Brainard and Doupe, 2002; Pfenning et al., 2014), and previous research has shown vocal imitation in fairy-wrens (Greig et al., 2012; Colombelli-Négrel et al., 2012). Therefore, it is reasonable to conclude that superb fairy-wren fledgling song elements were learned. The finding that sons and daughters produced "unique" elements from mothers and social fathers is an exciting finding because few studies to date have tested if both sexes may be vocal tutors for young male and female birds. Yamaguchi (2001) showed that captive Northern cardinals learn multiple song types from same-sex and opposite-sex vocal tutors, and Geberzahn and Gahr (2013) found that male and female blue-capped cordon-bleus also learn song from either sex, irrespective of the fact that males have larger syllable-type song repertoires than females. This is in contrast to other studies, which found that young birds showed a preference for learning vocalizations from same-sex vocal tutors (Wickler and Sonnenschein, 1989; Hausberger et al., 1995; Price, 1998).

Learning from two vocal tutors may increase an individual's chances of acquiring complex song. A wealth of research has shown that song complexity is an honest signal of male quality (Buchanan et al., 2004; Spencer et al., 2005; Schmidt et al., 2013), important for female mate choice (Catchpole, 1980; reviewed in Byers and Kroodsma, 2009). In superb fairy-wrens, different male song types predicted extra-pair fertilisations (Dalziell and Cockburn, 2008), and sexes had different element complexity (Kleindorfer et al., 2013). A theoretical framework for functions of female song complexity mostly focuses on resource defense (Cain and Langmore, 2015; Illes, 2015). Evidence for increased female fitness associated with complex female song comes from a few studies: older female alpine accentors (*Prunella collaris*) that sang more complex songs had larger clutch sizes (Langmore et al., 1996), and female European starlings with complex song had repeatable reproductive performance across years (Pavlova et al., 2010). Here we show that offspring had larger song element repertoires when exposed to element types of different vocal tutors, but we did not examine fledgling song complexity. We expect that fledglings with greater song complexity will have greater fitness.

The cooperative breeding system of the superb fairy-wren means that young birds interact with all group members (Mulder, 1995; Rowley and Russell, 1997). Social interactions with vocal tutors have been shown to facilitate song learning (Baptista and Petrinovich, 1984, 1986; Beecher et al., 1994; Beecher and Burt, 2004). Research on zebra finches (*Taeniopygia guttata*) found that social interactions influenced song tutor choice: young males preferred male vocal tutors that provided a greater amount of parental care (Williams, 1990), and chose fathers over unrelated males (Eales, 1987). The role of social interactions for the attention of young birds for particular adult vocal tutors remains to be tested in our system. All adults provisioned all offspring, and fledglings produced "shared" and "unique" parental element types. Although groups with helper males had on average one more "unique" element type per group, we did not find that fledglings of groups with helper males had a larger element repertoire than fledglings of groups without helper males. Helper males tend to be sons from a previous brood, and therefore these males also (largely) acquire their elements from the mother and social father. Perhaps the within-family element diversity is maintained rather than enhanced in the presence of helper males who may act as additional tutors.

Song Element Repertoire in Sons and Daughters

Previous research showed higher song complexity in adult males than females (Kleindorfer et al., 2013). It is possible that daughters lose element types with age, as we did not find sex differences in fledgling element repertoire size. Such a decrease in repertoire size with age has been found in female blue-capped cordon-bleus (Lobato et al., 2015). Males may have a longer sensory acquisition phase than females and acquire more element types over a longer timeframe, which is another explanation for why adult males have more complex song than females. Such a process has been proposed for Northern cardinals and blue-capped cordon-bleus (Yamaguchi, 2001; Geberzahn and Gahr, 2013; Lobato et al., 2015). All we can say at present is that we found no sex differences in fledgling song element repertoire, so therefore it is likely that sex differences emerge later in the superb fairy-wren.

Although sons and daughters sang element types of both parents, pairs with higher element diversity did not always have offspring with higher element diversity. This suggests that the development of complex song could be influenced by factors other than the element diversity of parents (Buchanan et al., 2003, 2004; Spencer et al., 2005; Schmidt et al., 2013). Rearing conditions can influence song learning and development. There is ample evidence for negative effects of developmental stress on song learning and complexity in male songbirds. Research into zebra finches found that developmentally stressed males had smaller HVC nuclei (area of brain associated with production of complex songs), and therefore lower song complexity (Buchanan et al., 2004; Spencer et al., 2005). Similarly, swamp sparrows (*Melospiza georgiana*) that received less food early in life had poor copy accuracy and a smaller repertoire size, suggesting that malnutrition affects song learning (Nowicki et al., 2002). Therefore, the observed variation in the average number of different elements across broods in this study could be explained by nutritional or environmental stress (Nowicki et al., 2002; Buchanan et al., 2004; Spencer et al., 2005), which we did not measure.

Exposure to song during development may have contributed to differences in the number of elements between broods. Offspring were exposed to both adult male and female song (Evans, unpublished data). However, we did not test for differences in song rate between adult males and females in the presence of fledged young in this study. Variation in song rates and subsequent exposure to song may influence song learning and copy accuracy (but see Hultsch et al., 1999). In a separate study of incubation calling in superb fairy-wrens, Kleindorfer et al. (2014) found that increased call rates resulted in higher vocal copy accuracy of chicks. Perhaps some of the variation in the number of chatter song elements produced by young birds is explained by the song rate of the parental birds (purported vocal tutors).

CONCLUSION

There is much interest in the function of female song, its complexity, and whether it is also subject to sexual selection. In this study we focused on the acquisition of song elements in sons and daughters in a system where both sexes produce solo song year round as adults. Sons and daughters produced vocal elements that included the same proportion of "shared" and "unique" elements between the pair male and female attending the nest. Given that male and female song elements were present in the song of the sons and daughters, we conclude that the diversity of element types in the combined "parental repertoire" was copied and produced by the entire brood, resulting in a "family vocal repertoire." This finding raises several new lines of inquiry into song patterns within families. We conclude with two exciting questions here: (1) Do social partners have assortative pairing for element diversity ("shared" and "unique") to increase song complexity in their offspring, and do offspring with higher vocal complexity have greater fitness? (2) Do social partners have disassortative pairing for "shared" elements to reduce inbreeding?

AUTHOR CONTRIBUTIONS

CE and SK designed the study, developed the methodology, performed the analysis, and wrote the manuscript. CE collected the data.

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Elaborate Mimetic Vocal Displays by Female Superb Lyrebirds

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Some of the most striking vocalizations in birds are made by males that incorporate vocal mimicry in their sexual displays. Mimetic vocalization in females is largely undescribed, but it is unclear whether this is because of a lack of selection for vocal mimicry in females, or whether the phenomenon has simply been overlooked. These issues are thrown into sharp relief in the superb lyrebird, *Menura novaehollandiae*, a basal oscine passerine with a lek-like mating system and female uniparental care. The spectacular mimetic song display produced by courting male lyrebirds is a textbook example of a sexually selected trait, but the vocalizations of female lyrebirds are largely unknown. Here, we provide the first analysis of the structure and context of the vocalizations of female lyrebirds. Female lyrebirds were completely silent during courtship; however, females regularly produced sophisticated vocal displays incorporating both lyrebird-specific vocalizations and imitations of sounds within their environment. The structure of female vocalizations varied significantly with context. While foraging, females mostly produced a complex lyrebird-specific song, whereas they gave lyrebird-specific alarm calls most often during nest defense. Within their vocal displays females also included a variety of mimetic vocalizations, including imitations of the calls of dangerous predators, and of alarm calls and song of harmless heterospecifics. Females gave more mimetic vocalizations during nest defense than while foraging, and the types of sounds they imitated varied between these contexts, suggesting that mimetic vocalizations have more than one function. These results are inconsistent with previous portrayals of vocalizations by female lyrebirds as rare, functionless by-products of sexual selection on males. Instead, our results support the hypotheses that complex female vocalizations play a role in nest defense and mediate female-female competition for breeding territories. In sum, this study reveals elaborate female vocal displays in a species widely depicted as an example of sexual selection for male extravagance, and thus highlights the hidden complexity of female vocalizations.

Keywords: female song, female ornaments, lyrebird, *Menura novaehollandiae*, nest defense, sexual selection, vocal mimicry

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INTRODUCTION

Songbirds have so been named for their elaborate and aesthetically pleasing vocalizations, and until recently (Odom et al., 2014) their 'songs' were assumed to be primarily the result of selection on males for attracting mates or repelling rivals (Catchpole and Slater, 2008). However, evidence has been accumulating that not only do female oscine passerines regularly sing, but also that their vocalizations play an important role in mediating female fitness. Compellingly, a recent analysis showed that female song is an ancestral trait in the oscine passerines (Odom et al., 2014), adding weight to earlier suggestions that the contemporary research focus on species that have little or no song in females has led to a distorted perspective on the sex-specificity of elaborate vocalizations in birds (Langmore, 1998; Hall, 2004; Riebel et al., 2005). Thus, there is now a strong impetus to understand the role of vocalizations in female oscine passerines to address this research imbalance.

While interest in female vocalizations is growing, it is currently restricted to studies of species-specific song and calls (Dalziell et al., 2015). However, between 20 and 40% of songbirds regularly imitate the sounds in their environment, such as those made by other species including birds and mammals, as well as mechanical sounds (Dalziell et al., 2015). Some of the most complex songs produced by male oscines around the globe incorporate imitations of heterospecifics. Such 'vocal mimics' include European starlings *Sturnus vulgaris* (Eens, 1997), marsh warblers *Acrocephalus palustris* (Dowsett-Lemaire, 1979), northern mockingbirds *Mimus polyglottos* (Gammon and Altizer, 2011), Lawrence's thrush *Turdus lawrencii* (2010), and chorister robins *Cossypha dichroa* (Harcus, 1977). However, research into the mimetic vocalizations of females is conspicuously absent. Here we provide the first investigation into the mimetic and non-mimetic vocalizations given by females of one of the world's best-known and most versatile vocal mimics, the superb lyrebird, *Menura novaehollandiae*.

Superb lyrebirds are a large (~1 kg), basal oscine passerine, with a slow life-history and with all the hallmarks of a species where males are under strong sexual selection. Adult males (>6 years) possess a spectacular tail (Smith, 1999, 2004) that they exhibit during sophisticated coordinated song and dance performances to females (Dalziell et al., 2013). Song and dance displays are performed on display arenas known as 'mounds' a circular patch of cleared ground on the forest floor (Higgins et al., 2001). During the breeding season, a single male will construct and maintain several mounds on a territory, which he defends from other males in a lek-like mating system (Higgins et al., 2001; Dalziell, 2012). Females are more cryptic in appearance, lacking the ornate tail feathers possessed by males. A single female builds a nest and cares for a single young (Lill, 1979a), and during this time defends a foraging/breeding territory from other females (Higgins et al., 2001). Females, like males, are seasonally territorial, and will defend the same territory in successive breeding seasons (Higgins et al., 2001).

Male lyrebirds are highly accomplished vocal mimics and also produce a diverse array of lyrebird-specific songs; however, the vocalizations of female lyrebirds are almost completely unknown. Male advertisement song is loud and complex, with 70–80% comprising highly accurate imitations of the vocalizations of sympatric species of birds (Robinson and Frith, 1981; Smith, 1988; Robinson and Curtis, 1996; Zann and Dunstan, 2008; Dalziell and Magrath, 2012). Males sing extensively during mid-winter when most eggs are laid (reviewed in Higgins et al., 2001), and vocal displays immediately precede copulations—characteristics consistent with a sexually selected trait. In contrast, female vocalizations are only given cursory mention—if at all—and are usually described as rare (Higgins et al., 2001) or performed less "skillfully" than by males (Lill, 2004). There are no descriptions of lyrebird-specific 'song' sung by females, but while some authors specify that females do not produce lyrebird-specific song (Lill, 2004) there are also descriptions of female lyrebirds that 'sang' as they defend their nests (e.g., Reilly, 1970, p. 69). There are, however, several anecdotal reports of breeding female lyrebirds imitating other species of bird (e.g., Cook, 1915; I. W., 1944), particularly when humans approach the nest or newly fledged young (e.g., Kitson, 1905; Robinson, 1975). Robinson (1975) dismisses these mimetic vocalizations as a "displacement activity" (Robinson, 1975, p. 24; *sensu* Delius, 1967). However, this conclusion seems premature given the absence of any systematic study on female lyrebird vocalizations. Moreover, the propensity for female lyrebirds to vocalize may have been underestimated because adult females (>4 years) have almost identical plumage to immature males (3–5 years) so that vocalizing females can easily be mistaken for vocalizing young males.

In this study we examined the structure and context of the vocalizations of female superb lyrebirds. Our preliminary observations indicated that, like males, breeding female lyrebirds regularly call, sing, and imitate other species. If female vocalizations are functionless by-products of selection for song and complex mimetic vocalizations in males, then their acoustic structure should not vary predictably with ecological context, and should be similar to that of males. Alternatively, if female vocalizations are the result of distinct selective forces acting on females, then they should vary predictably with female-only activities, such as female-female competition for foraging/breeding territories and nest defense. Furthermore, the acoustic structure of female vocal displays should differ from that of males. Here we examine these predictions by the analysis of recordings of wild female lyrebirds made in three different ecological contexts: (i) foraging, (ii) nest defense, and (iii) visits to courting males. We then discuss how female vocalizations differ from those reported in males.

METHODS

We studied nesting female superb lyrebirds in the Jamison Valley (−33.76°, 150.33°) within the Blue Mountains National Park in New South Wales, Australia. The valley is partially enclosed by sandstone cliffs and contains a mosaic of different habitats, but lyrebirds predominantly occupy sections of wet sclerophyll forest, and patches of temperate rainforest found along creek lines and in the shelter of the cliffs.

We recorded female lyrebirds between June and September in 2014 and 2015. The breeding season begins in winter and comprises two distinct parts. From May until mid-July, eggs are laid and adult male lyrebirds display and sing extensively. When fertile, a female lyrebird will leave her own territory and approach a male lyrebird on his territory. The male will then entice the female to one of his display mounds, where he displays (Higgins et al., 2001). A female may visit several males on these display evaluation forays before mating (Lill, 1979a), which takes place on the display mound itself (Higgins et al., 2001). By late-July most adult males have begun their annual tail molt, when they replace all their tail feathers at once. At this time male display activity rapidly decreases and males are less territorial. By this second stage of the breeding season, females are incubating and defending territories from other females. Females incubate their clutch (of one egg) for 6–7 weeks (Reilly, 1970; Lill, 1979a,b). The chick remains in the nest for a further 6–7 weeks and is brooded by the female. Once the chick fledges, the female's territory boundaries appear to dissolve but the fledgling will remain with its mother for several months and often until she nests again, which is usually in the following winter (AHD et al., unpublished data). While females and immature males can have identical plumage, during the incubation and nestling breeding stages the long tails of nesting females become bent sideways from sitting inside their dome-shaped nest. Since, in this species males never incubate (Reilly, 1970; Lill, 1979a), a bird with such a bent tail can be reliably identified as a breeding female.

Recordings

To examine the structure and context of the vocalizations of female superb lyrebirds, we recorded breeding females during three ecological contexts: foraging, nest defense, and courtship.

Foraging and Nest Defense

Foraging

We observed and sound-recorded 10 individual foraging females known to be incubating or brooding (recordings were 18–122 min, $\bar{x} = 54$ min). For three females for which the nest was known, we followed the individual from the moment she left her nest in the morning for up to 2 h. Other females were recorded in the vicinity of known nests or were recorded opportunistically. All recordings of foraging forays took place between 7 a.m. and 12 p.m. The female lyrebirds that were foraging were clearly habituated to human presence (for example, some continued foraging as members of the public ran past within 5 m) and thus it is unlikely they responded to the observer as a threat. We also watched for the presence of predators as these might confound our recordings. Accordingly, we excluded from our analysis the final portion of a recording of one female after detecting the presence of a gray goshawk, *Accipiter novaehollandiae* (a predator of lyrebirds: Lill, 1980).

Nest defense

We observed and sound-recorded eight individual females when we approached nests to band and measure nestlings (recordings were 8–42 min, $\bar{x}$ 21.6 min). When humans come close to an active lyrebird nest, female nest owners defend their nest by closely approaching the intruder and performing visual and vocal threat displays (sometimes leading to physical attack). These responses are consistent with those observed during natural nest disturbances (**Supplementary Video S1**). Thus, we are confident that our presence at the nest simulated a nest predator (see also Frid and Dill, 2002). Focal females were chosen randomly but we excluded from our analysis one recording of a female because for the majority of the recording she vocally interacted with another lyrebird. This prevented vocalizations from being attributed to specific individuals and rendered the context qualitatively different from the other recordings of a single female defending her nest.

Sound recordings of females foraging and defending their nest were obtained with a hand-held Sennheiser ME 66/K6 shotgun microphone and either a Maranz PMD 661 or a Roland R-26 digital recorder, set to sample at 48 kHz and 24 bits. We were careful to include in our analysis only sounds that originated from the focal lyrebird. In practice this was straightforward—the vocalizing lyrebird was in view or temporarily out of view in a known location during recordings. While model species were very rarely close by, we noted any other species viewed during the recording to identify cases where it was not possible to reliably assign the vocalizations to the lyrebird or the model. Accordingly, of 250 mimetic vocalizations identified among recordings, we excluded two vocalizations from our analysis because a model species was close by (a white-browed scrubwren, *Sericornis frontalis*, in both cases).

Courtship

We examined the role of female vocalizations during sexual interactions using 27 different videos of 15 different males interacting with females on display mounds. To record courtship displays, we placed motion-sensitive camera traps (Bushnell NatureView HD Max) at male display mounds. Once triggered, cameras recorded 60 s of video (resolution 1920 × 1080 p @ 30 fps) and sound (44.1 kHz, compressed as MP3 @ 64 kbps) before retriggering.

Acoustic Classification of Female Lyrebird Sounds

We found that, like males (Zann and Dunstan, 2008; Dalziell, 2012), female lyrebirds could produce a bout of continuous but varied sound. However, these bouts could be broken up into vocalizations. We formally defined a 'vocalization' as a continuous sequence of sound containing elements that were all of one of the following four mutually-exclusive 'types' lyrebird-specific 'alarm calls', lyrebird-specific 'whistle song', 'mimetic vocalizations', or 'unknown' (details below). Vocalizations were considered finished when the bird was silent for two or more consecutive seconds (thus, within a vocalization silent pauses could be no longer than 2.0 s). We identified these types audio-visually, by listening to recordings and visually inspecting sonograms in Raven Pro 1.5 (Cornell Lab of Ornithology, Ithaca).

Lyrebird-Specific Vocalizations (Alarm Calls and Song)

Lyrebird-specific vocalizations have not been described in females; therefore, to identify lyrebird-specific alarm calls and whistle songs we referred to our recordings of male lyrebird vocalizations from earlier studies (Dalziell, 2012; Dalziell and Magrath, 2012; Dalziell et al., 2013), published sonograms of males (Powys, 1995; Robinson and Curtis, 1996; Higgins et al., 2001), and a single sonogram of a female vocalization (Higgins et al., 2001). Detailed written descriptions also informed our identification of lyrebird-specific alarm calls (reviewed in Higgins et al., 2001). This conservative approach increased our confidence that all sounds classified as lyrebird-specific were in fact correctly classified (thus minimizing type I errors).

Mimetic Vocalizations

To identify mimetic vocalizations, we compared recordings of lyrebirds to recordings of putative model species. Recordings of models were sourced from our own recordings, the Macaulay Library of Animal Sounds (Cornell Lab of Ornithology), the open source web-based archive '*Xeno-canto*' (Xeno-canto Foundation), and commercial recordings (Plowright et al., 2007; McNabb, 2008; Morcombe and Stewart, 2014). Both authors were involved in the audiovisual identification of mimetic vocalizations.

Mimetic vocalizations were further subdivided by the criterion that all elements within a single vocalization were imitations of the same model species and were of the same element type (e.g., all elements within a single mimetic vocalization could be either imitations of alarm calls or song elements but they could not be both). These subdivisions were then allocated to one of three 'mimetic categories' based on the possible function of the heterospecific sounds for the mimicking lyrebird: (i) *Predator* imitations were imitations of the calls or songs of likely predators of adults and/or nests of lyrebirds, (ii) *Alarm* imitations were imitations of the alarm calls of birds likely to be harmless to lyrebirds, and (iii) *Non-alarm* imitations were imitations of the songs and other non-alarm vocalizations of birds harmless to lyrebirds. All mimetic categories were mutually exclusive.

Unknown Vocalizations

Of all vocalizations in our dataset ($n = 1135$), 9.9% were not classifiable as lyrebird-specific or as mimetic, and were classified instead as 'unknown.' Some of these unknown vocalizations may have been female lyrebird-specific vocalizations that were wholly unlike those of male lyrebirds so that no published examples were available, whereas others may have been imitations of models that we were unable to identify.

Statistical Analysis

To examine how the structure of vocalizations varied with context, GLMMs were constructed using GenStat 18 (VSN International, Hemel Hempstead, U.K.) and effects were estimated using restricted maximum likelihood (REML).

Vocalization Types vs. Ecological Context

To test how the different vocalization types varied with ecological context, we constructed a GLMM with a *Poisson* distribution and a log link function that included 'proportion of time vocalizing' (i.e., the total duration of each vocalization type per recording divided by the duration of the recording) as the dependent variable; 'vocalization type' (i.e., 'alarm call,' 'whistle song,' 'mimetic vocalization,' 'unknown'), 'ecological context' ('foraging,' 'nest defense'), and their interaction term as fixed effects; and 'Female ID' as a random factor. A dispersion parameter was estimated to control for over-dispersed residuals [dispersion parameter (Sigma^2) = 0.020, SE = 0.0039]. We evaluated the explanatory power of predictive factors using a backwards-stepwise selection procedure.

Temporal Association between Mimetic and Lyrebird-Specific Vocalizations

To examine the temporal association between mimetic and lyrebird-specific vocalizations, we compared the expected vs. observed probability distributions of vocalization types that immediately followed a mimetic vocalization.

To ensure that we only compared vocalizations that were closely associated in time, we included only vocalizations that followed within 8.4 s of a mimetic vocalization (corresponding to 75th percentile of the length of pauses between vocalizations within a recording). From these, we calculated the number of expected vocalizations of each vocalization type (i.e., 'alarm call,' 'whistle song,' 'mimetic vocalization,' 'unknown'), for each female, given the total number of mimetic vocalizations that she produced and the frequency of those types in the recordings. Thus, each female was given eight 'vocalization counts' consisting of four 'observed' and four 'expected' counts (one each for each vocalization type).

Next, we fitted a GLMM with a *Poisson* distribution and a log link function that included 'vocalization count' as the dependent variable; 'vocalization type,' 'observed vs. expected,' and their interaction term as fixed effects; and 'Female ID' as a random factor. A dispersion parameter was estimated to control for over-dispersed residuals [dispersion parameter (Sigma^2) = 2.276, SE = 0.4070]. Since the expected vocalization type frequencies were calculated from the data, we penalized the model with one additional residual degree of freedom.

Here, the key prediction is that if mimetic vocalizations are more closely associated with a particular vocalization type than expected by chance, then the interaction term should be significant.

Mimetic Vocalization Categories vs. Context

Finally, we examined whether females modified their 'mimetic categories' of mimetic vocalizations ('predator,' 'alarm,' 'non-alarm') according to the ecological context ('foraging' vs. 'nest defense') in which they were produced. To do this, we fitted a GLMM with a *Poisson* distribution and a log link function that included the number of mimetic vocalizations produced by females as the dependent variable; 'context,' 'mimetic category,' and their interaction term as fixed effects; and 'Female ID' as a random factor. A dispersion parameter was estimated to control for over-dispersed residuals [dispersion parameter (Sigma^2) = 1.572, SE = 0.3900]. One individual female was excluded from the final model because she exerted a disproportionate influence on the model predictions; however, results were qualitatively the same and significant whether or not this female was included.

RESULTS

Structure

Female superb lyrebirds regularly produced complex and highly varied vocal sounds (**Figure 1**; **Supplementary Audio S2**) comprising both lyrical pure-tone sequences of song-like elements as well as explosive and arresting calls. Within these long and varied bouts, we identified three broad types of vocalizations: (i) characteristic alarm calls unique to lyrebirds (**Figure 2**), (ii) a lyrebird-specific multi-element song (**Figure 3**), and (iii) imitations of other species of bird (**Figure 4**) (henceforth: 'alarm calls,' 'whistle songs,' and 'mimetic vocalizations' respectively: details below).

Lyrebird-Specific Alarm Calls and Whistle Songs

Lyrebird-specific alarm calls were highly distinctive in our recordings and were produced by most females (12/15). We classified three sounds as alarm calls (**Table 1A**; **Figure 2**): high-pitched and often ear-splitting 'scream' calls ('intense alarm call,' sonogram N, p. 162, Higgins et al., 2001); guttural alarm calls that were low-pitched and broadband; and a two-note alarm call (similar to the 'aw-kok' call: Smith, 1988). All three calls are associated with multiple lyrebird populations (AHD, personal observation) and are similar to previous written descriptions (reviewed in Higgins et al., 2001).

Unexpectedly, female lyrebirds also regularly produced a species-specific melodic vocalization (13/15 females: **Figures 3A–C**, **Table 1A**). These vocalizations were very similar in structure to the 'whistle songs' described for male lyrebirds (Zann and Dunstan, 2008; Dalziell, 2012; also called 'territorial song': Powys, 1995; Higgins et al., 2001) (**Figure 3D**). Thus, we name these female vocalizations 'whistle songs.'

Mimetic Vocalizations

Mimetic vocalizations were recorded in 11 of 15 nesting female lyrebirds (**Table 1B**). Across individuals, females most commonly imitated the vocalizations of other avian species, particularly those of two known predators of birds—the collared sparrowhawk (*Accipiter cirrocephalus*) and gray goshawk—as well as the calls of a large harmless cockatoo, the yellow-tailed black-cockatoo (*Calyptorhynchus funereus*), and a variety of vocalizations of a colonial honeyeater, the bell miner (*Manorina melanophrys*) (**Figures 4A–E**). Across females, we identified imitations of 19 species of bird (**Table 1B**) and all were found within our study site. There was considerable variation among female lyrebirds in the proportion of vocalizations that we could identify as mimetic. Some individuals did not appear to produce any mimetic sounds at all, but in one individual almost 75% of all vocalizations were imitations of other species.

Within female lyrebird vocalizations, we did not identify any imitations of sounds of human origin, despite the regular presence of people in the study site. Females could imitate non-vocal sounds, as we clearly identified imitations of bird wing beats, and one lyrebird appeared to imitate the distinctive squeak sound of two trees rubbing together in high winds, although in this case we were unable to obtain a recording of the suspected model sound.

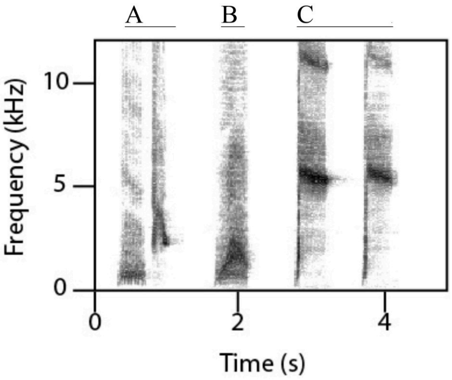

FIGURE 2 | Lyrebird-specific alarm calls. The three alarm calls most commonly produced by female lyrebirds are: **(A)** a two-note alarm call ('aw-kok'), **(B)** a guttural alarm call comprising one or more elements (one element shown here), and **(C)** a scream-like call ('intense alarm call') comprising one or more elements (two elements shown here) (See **Table 1A** for the distribution of lyrebird-specific alarm calls among females).

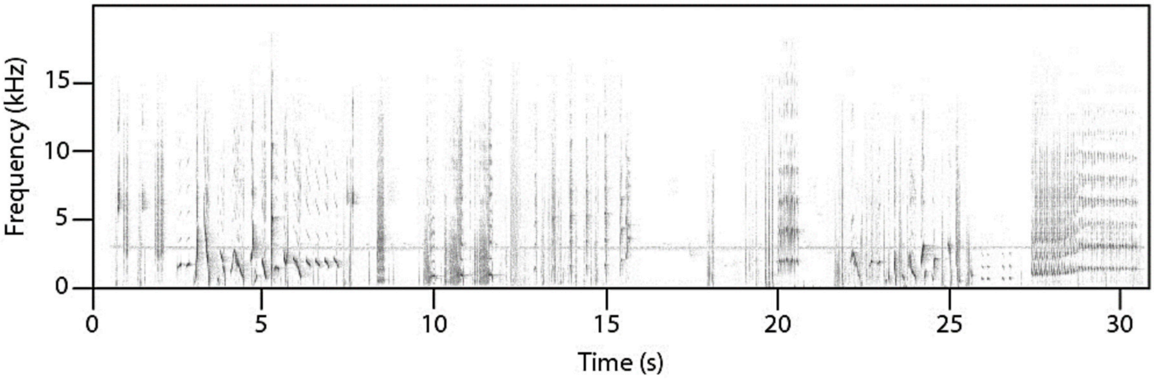

FIGURE 1 | A sample of an unedited recording of a female lyrebird vocalizing during nest defense. The apparent line at 3 kHz is the incessant song of a white-throated treecreeper (*Cormobates leucophaea*), all other sounds are produced by the female lyrebird (See **Supplementary Audio S2** for the audio file).

TABLE 1 | (A) Lyrebird-specific vocalizations in recordings of 15 adult female superb lyrebirds, (B) Mimetic vocalizations in recordings of 11 adult female superb lyrebirds (3 females we recorded did not produce mimetic vocalizations).

Vocalization type	Name	Female ID*
A		
Alarm	Scream	A,B,C,D,F,G,H,I,J,N
Alarm	Guttural alarm	A,B,D,F,K,M,N,D
Alarm	‘Aw-kok’	A,F,K,M,N
Song	Whistle song	A,B,C,D,E,G,I,J,K,L,M,N,O

Model species	Common name	Type of vocalization in model species	Mimetic category	Female ID*
B**				
Accipiter cirrocephalus	Collared sparrowhawk	Territorial call	Predator	A,C,F,K,M,N
Calyptorhynchus funereus	Yellow-tailed black-cockatoo	Non-alarm call	Non-alarm	A,C,F,K,L,M
Accipiter novaehollandiae	Gray goshawk	Territorial call	Predator	A,F,K,M,N
Manorina melanophrys	Bell miner	Mobbing call	Alarm mimicry	A,D,E,G,I
Sericornis frontalis	White-browed scrubwren	Mobbing call	Alarm mimicry	A,B
Acanthiza pusilla	Brown thornbill	Mobbing call	Alarm mimicry	C,N
Strepera versicolor	Gray currawong	Song	Predator	C,L
Strepera graculina	Pied currawong	Song	Predator	E,M
Manorina melanophrys	Bell miner	Song	Non-alarm	D
Manorina melanophrys	Bell miner	Unknown	Non-alarm	D
Platycercus elegans	Crimson rosella	Song	Non-alarm	C
Platycercus elegans	Crimson rosella	Alarm	Alarm mimicry	E
Rhipidura albiscapa	Gray fantail	Mobbing call	Alarm mimicry	C
Scythrops novaehollandiae	Channel-billed cuckoo	Non-alarm call	Non-alarm	C
Myiagra rubecula	Leaden flycatcher	Unknown	Non-alarm	C
Calyptorhynchus lathami	Glossy black-cockatoo	Non-alarm call	Non-alarm	C
Alisterus scapularis	Australian king-parrot	Alarm call	Alarm mimicry	C
Sericornis frontalis	White-browed scrubwren	Non-alarm	Non-alarm	C
Eopsaltria australis	Eastern yellow robin	Song	Non-alarm	C
Philemon corniculatus	Noisy friarbird	Unknown	Non-alarm	N
Psophodes olivaceus	Eastern whipbird	Song	Non-alarm	E
Ptilonorhynchus violaceus	Satin bowerbird	Song	Non-alarm	E
Oriolus sagittatus	Olive-backed oriole	Song	Non-alarm	C
Unknown	Wingbeats	NA	Not classified	C
Eucalyptus spp.	Squeaky tree	NA	Not classified	C

*The females that produced each vocalization type are individually identified by a letter (See **Figure 2** for sonograms of alarm vocalizations, and **Figure 3** for sonograms of song). ****B**: For each mimetic vocalization, we identified the species and the type of vocalization that was imitated (e.g., song, alarm call etc.). These two properties were used to classify mimetic vocalizations into three mimetic categories (column 3). Mimetic vocalizations are ordered by the number of different females that produced that sound (column 5) (See **Figure 4** for sonograms of a sample of mimetic vocalizations).*

Function and Context

Female lyrebirds regularly vocalized while foraging away from their nests ($n = 10$) and during nest defense ($n = 8$), with a trend toward a higher vocalization rate during the latter [2.3 ± 2.11 vs. 5.5 ± 3.90 s/min, respectively; Welch's ANOVA (for uneven SD): $F_{(1,10.4)} = 4.33$, $P = 0.063$]. However, female lyrebirds were completely silent when courting with males on display mounds ($n_{\text{interactions}} = 27$, $n_{\text{males}} = 15$), ruling out any function for elaborate vocalizations by females during sexual interactions with males.

Female lyrebirds produced different types of vocalizations in different contexts (**Figure 5**, **Table 2**). Females sang whistle songs while foraging but rarely produced mimetic vocalizations or lyrebird-specific alarm calls. However, during nest defense females produced mostly lyrebird-specific alarm calls and mimetic vocalizations. Vocalizations that we could not classify were produced at similar rates to mimetic vocalizations across contexts, suggesting that such sounds were either imitations of sounds we were unfamiliar with, or poor imitations.

Mimetic vocalizations were non-randomly associated with vocalization types (**Figure 6**, **Table 3**). Mimetic vocalizations were more likely to be followed by mimetic vocalizations but were less likely to be followed by lyrebird-specific alarm vocalizations, compared to the expected frequencies of vocalization types.

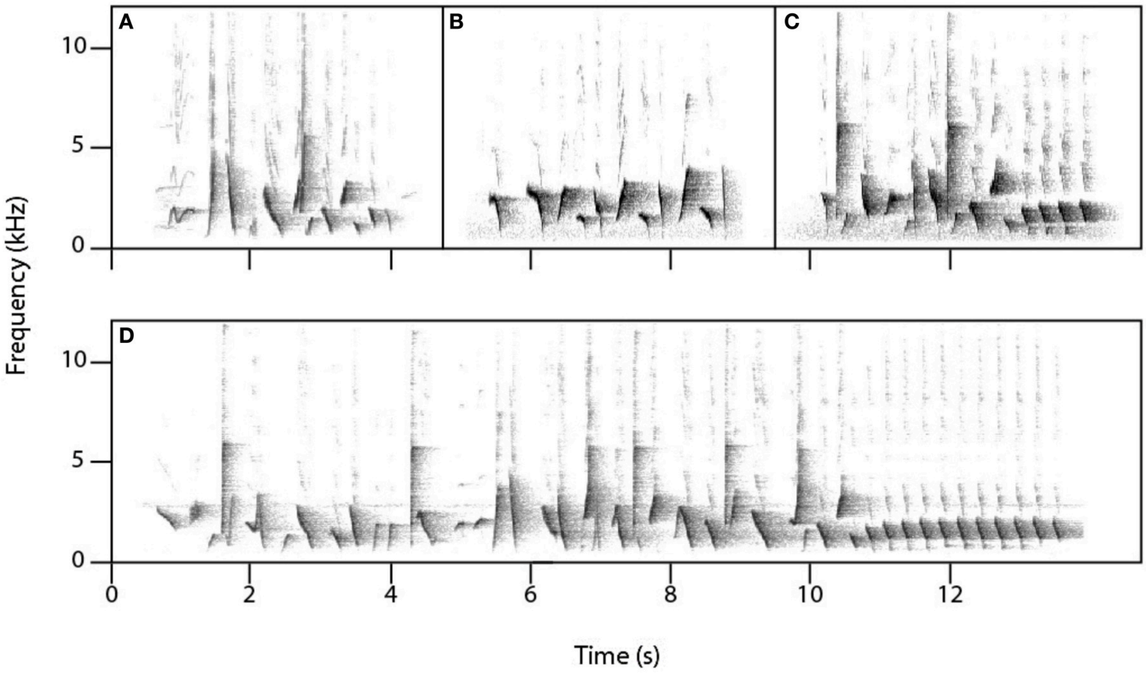

FIGURE 3 | Lyrebird-specific ‘whistle song’ as performed by three females (A–C) and a male (D).

TABLE 2 | Results from a generalized linear mixed model relating the proportion of time (of total recording time) females produced each of the four vocalization types (mimetic vocalizations, lyrebird-specific alarm, lyrebird-specific song and unknown), and ecological contexts (foraging forays vs. nest defense) (See also Figure 5).

Effects		Estimate	SE	Approx. F-statistic	Numerator d.f.	Denominator d.f.	*P*
Random	Female	0.369	0.266				
Fixed	Constant	−5.13	0.602				
	Vocalization type			1.17	3	53.0	0.331
	Context			3.76	1	39.4	0.060
	Vocalization type* Context			3.05	3	51.0	**0.037**

Significant P-values (<0.05) are highlighted in bold.

Finally, there was a difference in the *composition* of mimetic vocalization categories between contexts (**Figure 7**, **Table 4**). During nest defense females imitated all three mimetic vocalization types; however, during foraging they imitated the songs and calls of predators, while rarely imitating harmless model species.

DISCUSSION

Nesting female superb lyrebirds regularly produced complex vocalizations comprising a variety of species-specific sounds as well as accurate vocal imitations of other species. While preliminary, our results are consistent with a nest defense function for lyrebird-specific ‘alarm’ calls, and a territory defense function for the whistle song. Mimetic vocalizations likely play a role in both contexts but may have more than one function. These results contrast with prevailing notions that female lyrebird vocalizations are rare (Higgins et al., 2001) and of secondary importance (Robinson, 1975) to those of males. Instead, these results highlight the hidden complexity of female vocalizations in oscine passerines.

Lyrebird-Specific Vocalizations

Distinctive lyrebird-specific alarm calls formed a substantial part of the vocal repertoire of females, but were more strongly associated with nest defense than with foraging, consistent with a nest defense function. In lyrebirds, the burden of nest-defense is carried solely by the female. Offspring survival is an essential component of inclusive fitness and therefore nest defense strategies are likely to be under strong selection in birds with few, single-egg breeding attempts, like lyrebirds.

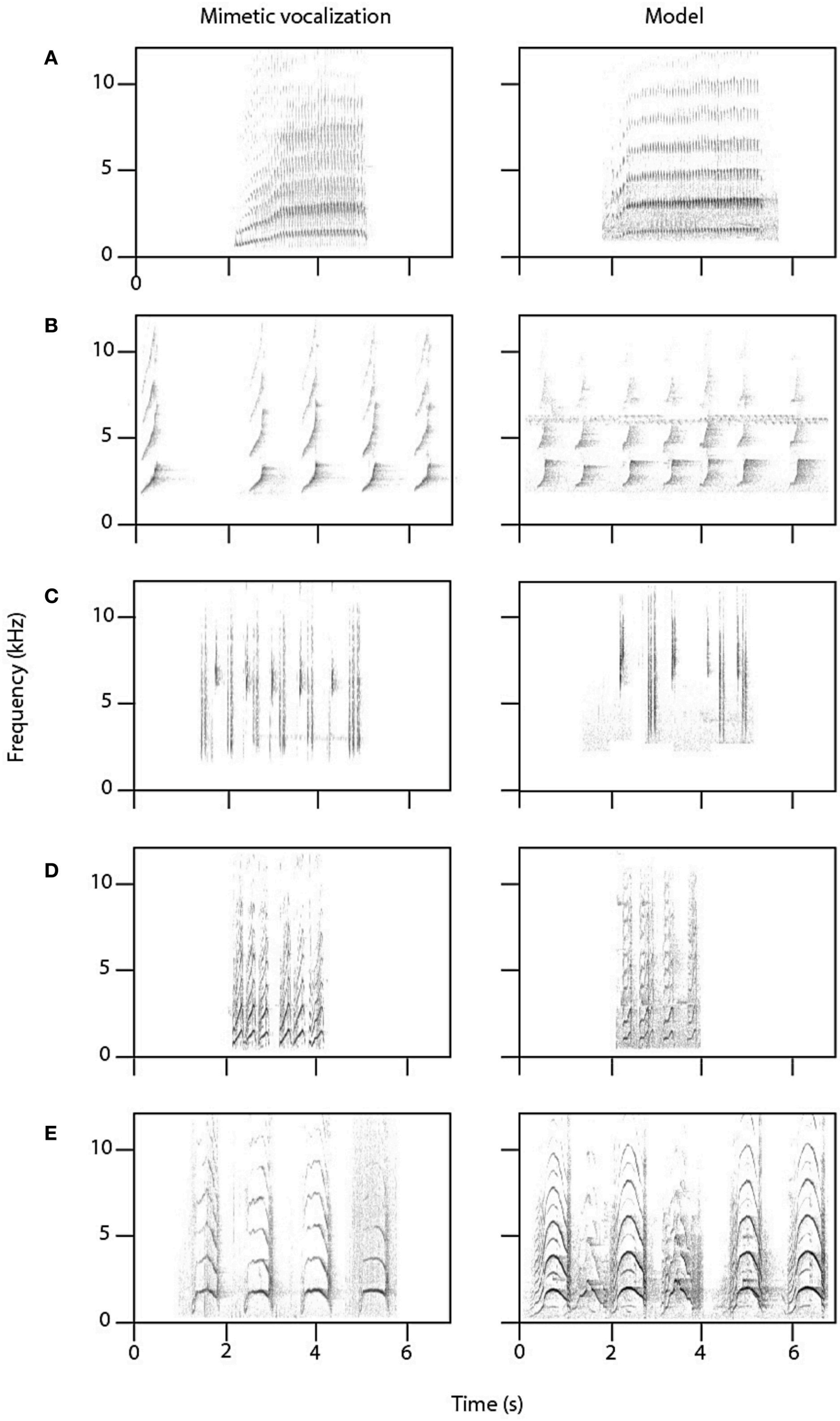

FIGURE 4 | Mimetic vocalizations by female lyrebirds (left-hand column) of five species of bird with a recording of the model species (right-hand column). (A) Collared sparrowhawk (*Accipiter cirrocephalus*); **(B)** gray goshawk (*Accipiter novaehollandiae*); **(C)** white-browed scrubwren (*Sericornis frontalis*); **(D)** bell miner (*Manorina melanophrys*); **(E)** yellow-tailed black-cockatoo (*Calyptorhynchus funereus*). Model credits: **(A)** Stewart (2001) Macaulay Library; **(B)** Anderson (2012) *Xeno-canto* CC; **(C)** Åberg (2012) *Xeno-canto* CC; **(D,E)** own data.

TABLE 3 | Results from a generalized linear mixed model comparing the observed frequency of different ‘types’ of vocalization (mimetic, lyrebird-specific alarm, whistle song, and unknown) that followed a mimetic vocalization, and the expected distribution of types calculated from the overall frequencies of each type within recordings (See also Figure 6).

Effects		Estimate	SE	Approx. F-statistic	Numerator d.f.	Denominator d.f.	*P*
Random	Female	1.27	0.680				
Fixed	Constant	1.03	0.432				
	Vocalization type			5.97	3	62.5	**0.001**
	Expected vs. Observed			0.01	1	61.5*	>0.9
	Vocalization type* Expected vs. Observed			5.20	3	61.5*	**<0.01**

Significant P-values (<0.05) are highlighted in bold.
An additional residual degree of freedom is associated with the calculation of the ‘Expected vs. Observed’ factor.

TABLE 4 | Results from a generalized linear mixed model of the number of different mimetic vocalizations per minute by context (Foraging vs. nest defense) and the category of mimetic vocalization (predator, alarm, or non-alarm) (See also Figure 7).

Effects		Estimate	SE	Approximate F statistic	Numerator d.f.	Denominator d.f.	*P*
Random	Female	3.38	1.68				
Fixed	Constant	−2.00	0.856				
	Context			0.94	1	43.5	0.337
	Mimicry type			7.67	2	32.5	**0.002**
	Context*Mimicry category			4.89	2	32.5	**0.014**

Significant P-values (<0.05) are highlighted in bold.

Accordingly, female lyrebirds vigorously defend their nests (**Supplementary Video S1**), closely approaching and sometimes physically attacking avian nest predators or humans near the nest (AHD and JAW unpublished data; Reilly, 1970; Lill, 1980). The conspicuousness and noxiousness of lyrebird alarm calls given during these events is often remarked upon (Higgins et al., 2001), and while it is likely that they play a role in nest defense, as they do in other bird species (Caro, 2005), it is as yet unclear how these calls reduce depredation.

Females regularly sang a loud, multi-element species-specific song analogous to the male ‘whistle song’ (Powys, 1995; Robinson and Curtis, 1996; Zann and Dunstan, 2008). Our results are consistent with a territory defense function for the whistle song, as females most often produced whistle songs while foraging away from the nest, and appeared to counter-sing to conspecifics—a common territorial defense behavior in other species (Catchpole and Slater, 2008). Studies of marked female lyrebirds show that they are territorial when breeding (e.g., Kenyon, 1972). Females can engage in female-female combat “just as aggressively as males” (p. 153, Higgins et al., 2001) and even destroy the nests of neighbors (Reilly, 1970). Given this backdrop of intra-sexual competition for nesting territories it is likely that the whistle song functions in the defense of breeding territories, as has been shown for females in other bird species (reviews: Langmore, 1998; Hall, 2004, 2009; also Brunton et al., 2008; Geberzahn et al., 2010; Krieg and Getty, 2016).

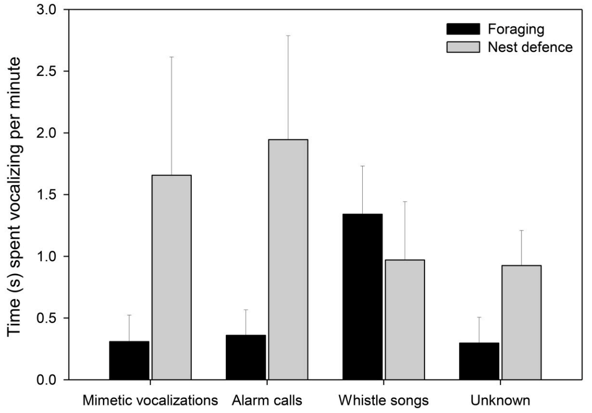

FIGURE 5 | The time (seconds ± SE) that females spent producing the four vocalization types (x-axis) in the foraging (black bars; $n_{\text{female}} = 10$) and nest defense contexts (gray bars; $n_{\text{female}} = 8$). Means and standard errors were calculated from raw data (See **Table 2** for details of the model).

Mimetic Vocalizations

Female lyrebirds imitated a range of different sounds in their environment but most commonly those associated with alarm, such as the mobbing alarm calls of harmless species of bird and the calls of dangerous predators. Females imitated during both nest defense and foraging but imitated more during nest defense, suggesting that mimetic vocalizations function in an antipredator context. Intriguingly however, there was a difference in the *composition* of the mimetic repertoires used in these contexts, with imitations of heterospecific alarm calls appearing more frequently during nest defense, whereas predator call imitations occurred similarly often in both the contexts. The predator calls that were imitated by most females were those of collared sparrowhawks and gray goshawks. These *Accipiter* spp. are known to attack adult female lyrebirds (Lill, 1980) and are therefore also likely predators of fledglings. It is tempting to speculate that by

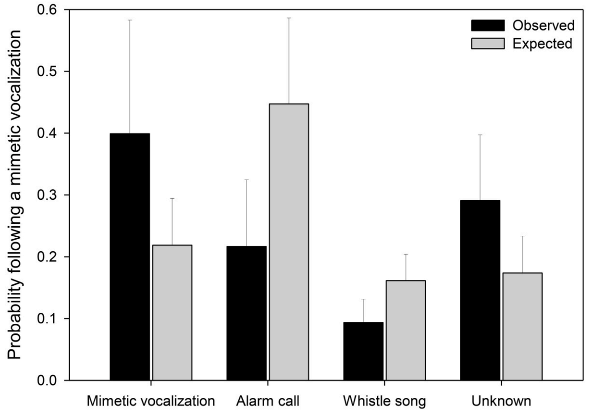

FIGURE 6 | The observed and expected probabilities that a vocalization subsequent to a mimetic vocalization was of a particular type (x-axis). Observed values (black bars) represent the average proportions of vocalizations (±SE) by type subsequent to a mimetic vocalization ($n_{\text{mimicking female}} = 10$, $\bar{x}$ number of vocalizations = 20.3). Expected values (gray bars) represent the average proportions of vocalizations (±SE) by type ($n_{\text{female}} = 15$, $\bar{x}$ number of vocalizations = 75.6). Means and standard errors were calculated from raw data (See **Table 3** for details of the model).

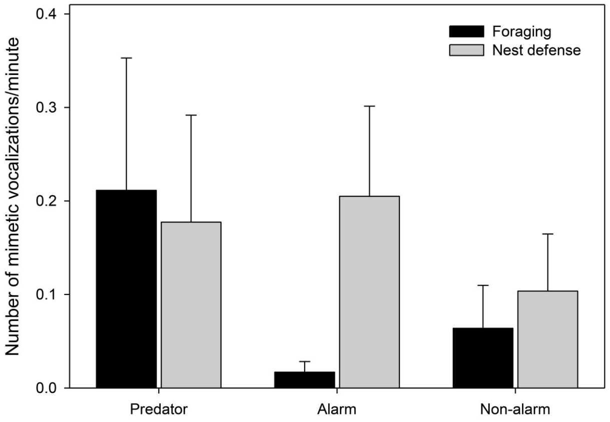

FIGURE 7 | The number of mimetic vocalizations observed per minute by category of mimetic vocalization (x-axis) during foraging forays (black bars) and nest defense (gray bars) ($n_{\text{mimicking female}} = 10$, $\bar{x}$ number of vocalizations = 20.3). Means and standard errors were calculated from raw data (See **Table 4** for details of the model).

imitating *Accipiter* hawks, females create the acoustic illusion that dangerous raptors are present ('crying wolf'—see also Flower, 2011; Flower et al., 2014; Igic et al., 2015), thus decreasing the attractiveness of their territories (Rechten, 1978). This intriguing hypothesis could be investigated experimentally by examining responses of females to playback of imitated predator calls.

Given the likely roles of mimetic alarm vocalizations and lyrebird-specific alarm calls in nest defense, we expected that these mimetic and non-mimetic alarms would be positively associated, as has been found in other avian mimics (Goodale and Kotagama, 2006). Therefore, it was somewhat surprising that females were *less* likely to follow a mimetic vocalization with a lyrebird-specific alarm call than would be expected by chance. One explanation for this negative association is that they represent alternative nest-defense strategies. This could reflect age-dependent vocal learning constraints that are thought to affect mimetic ability in males (Zann and Dunstan, 2008). If the ontogeny of female mimetic vocalizations is similar to that of males, the option of producing effective mimetic vocalizations may be available only to older females, whereas alarm calls would be produced predominantly by younger females that are less vocally developed.

Do mimetic vocalizations of female lyrebirds constitute functional mimicry? Vocalizations resembling those of heterospecifics can result from several processes, including chance, common ancestry, ecological convergence, or as by-products of vocal learning (reviewed in Dalziell et al., 2015). To be considered functional mimicry, however, the resemblance of a vocalization to a model should induce a behavioral change in receivers that provides a selective benefit the mimic (Dalziell et al., 2015). Clearly, in females not all mimetic vocalizations can be functional—it is difficult to imagine how receivers might respond to the sounds of a squeaking tree in a way that benefits a lyrebird! Nevertheless, the context-dependence of female mimetic vocalizations suggests that different types perform different functions, and so functional mimicry is plausible.

Are Female Vocalizations By-Products of Selection for Vocal Complexity in Males?

Overall, our findings are not consistent with the hypothesis that vocalizations by female lyrebirds are functionless by-products of selection for vocal complexity in males. First, females varied their vocalizations predictably with female-only activities, as whistle songs were primarily associated with a female-female competition context, and mimetic vocalizations with nest defense. During courtship females were completely silent. Males, however, produce whistle song and mimetic vocalizations together and almost exclusively during sexual advertisement and courtship (Powys, 1995; Robinson and Curtis, 1996; Zann and Dunstan, 2008; Dalziell, 2012). This sex-specific context-dependence supports the notion that different vocalizations perform functions important for mediating fitness in both females and males. Second, females regularly imitated species that are only rarely imitated by males, such as *Accipiter* hawks and bell miners (Higgins et al., 2001; Zann and Dunstan, 2008; Dalziell, 2012); conversely, only one female imitated an Eastern whipbird, *Psophodes olivaceus*, which is a heterospecific that almost all males regularly imitate (AHD and JAW, pers. obs.; Zann and Dunstan, 2008). Thus, mimetic vocalizations appear to mediate different fitness benefits for males and females. For example, males may employ mimetic repertoires that best demonstrate their vocal agility and are thus most effective at attracting mates, while females selectively mimic sounds that best deter predators or conspecific competitors. These sex differences in mimetic repertoires also show that females and males acquire mimicry through different routes:

females may directly copy model species or copy other females; whereas, males appear to learn their mimicry, at least in part, from other males (Robinson and Curtis, 1996; Higgins et al., 2001; Putland et al., 2006). In sum, both the functions and underlying mechanisms of elaborate vocalizations in lyrebirds appear to be different for both sexes, suggesting that males and females are under distinctly different selection pressures for vocal complexity.

General Implications for Elaborate Vocalizations in Female Songbirds

This study provides the first evidence of context-dependent female song in a species within the most basal clade of the oscine passerines. Identifying the ecological and life-history correlates of female song requires detailed study of song, particularly in species that diverged closest to the ancestral node (Odom et al., 2014). At present, little is known about female song in other early diverging species, with the exception of the *Maluridae* (Cooney and Cockburn, 1995; Hall and Peters, 2008; Colombelli-Négrel et al., 2010; Dowling and Webster, 2013; Cain et al., 2015). Therefore, it is not yet clear what aspects of song in lyrebirds have been retained since their ancestor diverged from the main branch of the oscines, and what features have since evolved. Nonetheless, this study strengthens the conclusion of Odom et al. (2014) that female song has a deep evolutionary history.

Our results are only partially consistent with the ecological and life-history correlates of female song that have hitherto been identified (reviewed in Langmore, 1998; Hall, 2004; Slater and Mann, 2004; Odom et al., 2014). Like other species with female song, lyrebirds are non-migratory (Price, 2009; Logue and Hall, 2014), and do not experience the seasonal extremes associated with the absence of song in females of northern hemisphere species (Slater and Mann, 2004). Our findings support the idea that complex song by females is more commonly associated with intra-sexual competition for ecological resources than with mate attraction (Cooney and Cockburn, 1995; Hall and Peters, 2008; Illes and Yunes-Jimenez, 2009; Cain et al., 2015, see also Tobias et al., 2012), but contradict suggestions of an association with convergent sex roles (Slater and Mann, 2004). Indeed, our study suggests that elaborate female vocal displays can evolve even in species with strong selection for male extravagance. It also shows that the degree of sexual dimorphism in acoustic display does not have to reflect the extent of sexual dimorphism in plumage. This should not be unexpected, given recent studies showing that visual and acoustic display traits can evolve independently within males (Greig and Webster, 2013; Mason et al., 2014) and across the sexes (Soma and Garamszegi, 2015). Clearly, patterns of sexual dimorphism in acoustic display cannot always be inferred from sex differences in visual displays or other life-history traits.

There are two key implications of our study for the understanding of song learning in oscine passerines. First, by showing that females, like males, are capable of learning accurately a variety of sounds in their environment, our study suggests that sexual differences may lie more in what is learned rather than in the ability to learn *per se* (Riebel, 2003). Second, our study shows that complex learned vocalizations in females can specifically occur in a nest-defense context, in addition to the more traditionally recognized song contexts of competition for mates and resources (Langmore, 1998; Catchpole and Slater, 2008). Thus, vocal learning and song production are not fully equivalent. There is evidence that learned mimetic vocalizations are associated with nest defense in several oscine families (Dalziell et al., 2015) and can provide adaptive benefits (Igic et al., 2015), suggesting that nest-defense may constitute an important source of selection for complex learned vocalizations. A greater focus on female vocalizations may thus provide key insights into both the mechanisms and functions of learned vocalizations more broadly.

In conclusion, this study reveals elaborate female vocal displays in a basal oscine passerine widely depicted as a textbook example of sexual selection for male extravagance. It shows that female vocalizations can vary adaptively with female-only activities in a manner distinctly different from that of males. This adds to the growing evidence that not only do female oscine passerines regularly sing, but also that their vocalizations play an important role in mediating female fitness (Langmore, 1998; Hall, 2004, 2009; Odom et al., 2014; Hall and Langmore, 2016[1]). This evidence forces us to reconsider entrenched notions about the function, ontogeny, and evolutionary history of complex vocalizations—both mimetic and species-specific—and recognize the need for a more female-inclusive approach for a full understanding of the causes and consequences of avian vocal displays.

ETHICS STATEMENT

All work was approved by the Western Sydney University Animal Care and Ethics Committee (#A10699) and the Cornell University Animal Care and Use Committee (2009-0105), and conducted under a Scientific Research Permit from the NSW National Parks and Wildlife Service, Office of Environment and Heritage (#SL101351).

AUTHOR CONTRIBUTIONS

Both authors contributed to all aspects of this paper.

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[1] http://journal.frontiersin.org/researchtopic/3139/female-song-fitness-costs-and-benefits

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: http://journal.frontiersin.org/article/10.3389/fevo.2016.00034

Supplementary Video S1 | Video of nest defense following a natural disturbance.

Supplementary Audio S2 | Unedited 30-s sound recording of a female lyrebird vocalizing during nest defense (supplementary to Figure 1).

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Kin Signatures Learned in the Egg? Red-Backed Fairy-Wren Songs Are Similar to Their Mother's In-Nest Calls and Songs

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Many vocal animals recognize kin using vocal cues, in territorial contexts and in rearing young, but little is known about the developmental and evolutionary mechanisms that produce vocal kin recognition systems. In the cooperatively breeding red-backed fairy-wren *(Malurus melanocephalus)*, females give specific "in-nest calls" while incubating their eggs. Elements from these calls are incorporated into chicks' begging calls, and appear to be used by parents for recognition. This is likely a result of an embryo's ability to learn the call elements in the egg. Here, we explore the idea that maternal vocal elements may be incorporated into offspring's adult songs, and serve as signatures of kinship, which would aid in kin recognition and benefit signaler and receiver. To investigate this hypothesis, we tested for similarities between maternal vocal elements (in-nest calls and songs) and songs of their adult offspring. We then determined whether offspring songs were more similar to the maternal vocalizations they heard only as embryos (in-nest calls), or maternal songs they heard throughout development, but mostly post-fledge (mothers' full songs). We used dynamic time warping to compare maternal vocal elements with elements in their offspring's adult songs. The elements of each offspring were more similar to the elements of their own mother than to the elements of any other female (but only for the average similarity score), suggesting they may serve as kin signatures. We also found that offspring song elements were more similar to their mother's song elements than to their mother's in-nest call elements. In addition, female in-nest call elements were more similar to their own song elements than to the song elements of any other female. Offspring that produced song elements highly similar to their mother's song elements also had song elements highly similar to their mother's in-nest call elements. Signals of kinship may function critically for survival and successful reproduction, by allowing an individual to allocate care and defense to kin and to avoid mating with genetic relatives. Understanding the processes that facilitate effective kin recognition, including development of kin signatures, helps us to understand how these crucial signaling systems may have evolved.

Keywords: kin signatures, female song, in-nest calls, embryonic learning, songbirds, *Malurus melanocephalus*

INTRODUCTION

Kin recognition can provide adaptive benefits to both senders and receivers of kinship signals. Receivers of such signals can provide care and feeding to related young, alert kin to resources and danger, and avoid mating with genetic relatives (Holmes and Sherman, 1983; Akcay et al., 2013). Senders can also benefit from reliably identifying themselves as kin, if this allows them to obtain nepotistic benefits and avoid inbreeding (Beecher, 1988). When senders benefit from being recognized, selection is expected to act directly on signal properties to enhance the reliability of kin signatures (Beecher, 1988).

Kin recognition systems have been described in different signaling modalities and across taxa (Porter, 1986; Hurst and Beynon, 2010). Despite this, we have a limited understanding of how kin signatures develop, or the mechanisms that underlie similarity among kin. Therefore, studies that elucidate how, when and from whom individuals acquire signatures that may be used for kin recognition are needed (Sharp et al., 2005). This information is an important piece in the evolutionary puzzle of how kin recognize one another, since senders and receivers can only benefit from recognition if individuals accurately acquire and produce signals produced by their kin (Waldman, 1987). We expect selection for ontogenetic mechanisms that minimize acquisition mistakes. Investigating these questions allows us to better understand the evolutionary underpinnings of kin recognition, a process that is critical for survival and successful reproduction across the majority of social animal species (Holmes and Sherman, 1983; Akcay et al., 2013).

In vertebrates, receivers recognize kin signatures most commonly by learning through direct association, rather than through innate recognition (Halpin, 1991; Caspers et al., 2013). In birds specifically, associative learning of kin signatures appears to be the primary kin recognition mechanism, especially in cooperatively breeding species (Sharp et al., 2005; Caspers et al., 2013; Amo et al., 2014), and vocalizations are the most common communication modality used for avian kin recognition (Beecher, 1988; Riehl and Stern, 2015). Vocal kin signatures have been described in many taxonomic groups (Price, 1998; McDonald and Wright, 2011; Knörnschild et al., 2012) and vocal kin recognition has been experimentally supported in several mammal (Rendall et al., 1996) and bird species (Price, 1999; Sharp et al., 2005; McDonald, 2012; Akcay et al., 2013). Although these avian studies have laid excellent groundwork, our understanding of the occurrence and extent of vocal kin recognition across avian groups remains incomplete. In addition, we know little about how young birds acquire vocal kin signatures and how these ontogenetic processes are shaped by selection. Gaining a more comprehensive understanding of vocal kin recognition systems, including their development and evolution, from the perspective of both senders and receivers, helps us to elucidate the signaling behaviors that can evolve via inclusive fitness benefits.

Here, we tested for the presence of kin signatures in the adult songs of the red-backed fairy-wren (*Malurus melanocephalus*) and investigated a potential mechanism of kin signature acquisition, as well as the timing of acquisition. In this species, females call to their eggs and nestlings while inside the nest using a particular "in-nest" call that they do not produce in other contexts (Colombelli-Négrel et al., 2016, **Figure 1**). Mothers begin calling at the onset of incubation and continue to call until day 5–6 post-hatching (Colombelli-Négrel et al., 2016). After hatching, young reproduce elements from these maternal calls, incorporating them into their begging calls (Colombelli-Négrel et al., 2016). This is likely a result of the embryos' ability to learn the elements from within the egg, which has been shown in the closely related superb fairy-wren (*Malurus cyaneus*) (Colombelli-Négrel et al., 2012, 2014). While feeding chicks at the nest, red-backed fairy-wren parents seem to recognize young that can imitate their maternal elements better and feed them preferentially (Colombelli-Négrel et al., 2016). This behavior is thought to have evolved in superb fairy-wrens because it decreases the cost of inter-specific brood parasitism (Colombelli-Négrel et al., 2012), an evolutionary pressure that red-backed fairy-wrens are also subject to (albeit less so, in our population, Colombelli-Négrel et al., 2016). Despite our understanding of kin signature use and recognition in red-backed fairy-wrens within the context of the nest, we do not yet know whether young incorporate maternal vocal elements (including both in-nest call and full song elements) into other vocalizations, such as their adult songs.

To investigate this, we compared maternal in-nest call and full song elements to elements present in the adult songs of their offspring. In addition, we determined if adult songs of offspring were more similar to the maternal vocalizations they heard only as embryos/nestlings (in-nest calls) or to the maternal songs heard throughout development and after fledging (mothers' full songs) in order to help us to understand when kin signatures may be acquired. We predicted that if kin signatures were present in red-backed fairy-wren songs, then offspring's song elements would be more similar to the elements of their own mother than to the elements of any other female. We additionally predicted that if this species used song elements to recognize kin, then the kin signatures individuals used for recognition as nestlings might be preserved in their adult songs, which might also suggest that kin signatures may be learned in ovo and/or soon after hatching.

METHODS

Field Methods

We studied a population of color-banded red-backed fairy-wrens near Herberton, Queensland, Australia (145°25′E, 17°23′S), which has been monitored continuously since 2003. The study site is located in open dry sclerophyl forest with tall eucalypt overstory and grass understory. Red-backed fairy-wrens breed cooperatively, with 34% of 1-year-old males remaining on their natal territory as auxiliary helpers (Varian-Ramos et al., 2010). A red-backed fairy-wren breeding group consists of a breeding male, breeding female and 0–3 auxiliary helpers (Varian-Ramos et al., 2010).

Females build domed nests low in the grassy understory and lay two to four (usually three) eggs per clutch. Females are solely responsible for building the nest and incubating the eggs, but all members of the group participate in feeding nestlings and fledglings (Varian-Ramos et al., 2010). Eggs usually hatch after 12 days of incubation and chicks fledge after 12 days in the nest. Fledglings remain dependent for approximately 40 days post-fledging and often remain on the territory through the winter with females, with breeding fledglings dispersing in the early spring prior to breeding. The reproductive biology of this non-migratory species is described in further detail by Webster et al. (2010).

In our study, group compositions, territory boundaries, and nest status were determined through daily observations of each breeding group and nest checks every other day. We monitored all breeding attempts by each group on the field site. On the sixth day after hatching, nestlings were banded with an aluminum band bearing a unique identifying number. We used this information to determine each bird's natal group and determined relatedness by linking offspring to the female that attended the nest, and constructing a social pedigree.

We recorded in-nest vocalizations from females in 7 nests, during the 2011-2012 breeding season (September–January). Vocalizations were recorded for 2 continuous hours per nest between days 10 and 12 of incubation. We recorded all vocalizations produced at the nest using either (1) an Olympus linear LS-10 handheld PCM recorder or (2) a Wildlife Acoustics Song Meter SM2 Autonomous recording unit (Wildlife Acoustics Inc., Concorde, MA, USA), both with a sample rate of 24 KHz in 16 bit PCM format. Both recorders had integrated microphones and were placed directly under the nest as close as possible without causing disturbance (usually 20–30 cm). All recordings were done in the morning between 0500 and 1100 h.

Songs of adult offspring were recorded in the breeding seasons of 2012-2013 and 2013-2014 during the pre-dawn chorus (Dowling and Webster, 2016). Each adult offspring was at least 1 year old and breeding independently at the time of recording. Independent breeders had established their own mate and a breeding territory; independently breeding females typically disperse several territories away from their natal territory, whereas males typically settle close to their natal territory (Varian-Ramos et al., 2010). Songs of adult offspring and full songs of mothers were recorded with a Marantz PMD 661 solid-state digital recorder (D&M Professional, Itasca, IL, U.S.A.) at 96 kHz sampling rate, 24-bit depth, connected to a K6 power module and a ME67 shotgun microphone capsule (Sennheiser Electronic Corporation, Old Lyme, CT, U.S.A.) with a Rycote softie windshield and mount. Individuals were recorded mostly during the pre-dawn chorus (Dowling and Webster, 2016). In the first comparison, we compared in-nest call elements of 7 mothers to adult song elements of 8 of their adult offspring (6 male, 2 female). Our sample of adult offspring included one offspring each for 6 mothers (5 male, 1 female), and two offspring for one of the mothers (1 male, 1 female). In the second comparison, we compared song elements of 5 mothers to adult song elements of 5 of their adult offspring (one offspring for each mother, 4 male, 1 female). In the third comparison, we compared 5 mother's in-nest call elements to full song elements from those same 5 mothers.

We transferred all recordings onto an Apple MacBook Pro (Apple Corporation, U.S.A) and spectrograms were created in RavenPro 1.5 (Cornell Laboratory of Ornithology, Ithaca, NY, USA, 16-bit sample format; discrete Fourier transform (DFT) = 512 samples; frequency resolution = 124 Hz; time resolution = 11.6 ms; frame overlap = 50%). Recordings of female in-nest calls were band-pass filtered so that only the spectral area between 1000 and 17,800 Hz remained, which removed background noise but left the signal intact. Recordings of songs of adult offspring and mothers were band-pass filtered so that only the spectral area between 2400 and 17800 Hz remained, again removing background noise but leaving the signal intact.

Element Comparisons

We selected one exemplar of each element type in each individual's repertoire for maternal in-nest calls, maternal songs, and their offspring's adult songs. Element types were classified for maternal songs and offspring adult songs using element classification methods described in Schwabl et al. (2015). In short, we used JMP 11.0 (SAS Institute Inc., Cary, NC, U.S.A.) to classify elements into groups of element types using normal mixtures clustering. From this, we identified an optimal number of clusters (i.e., element types) using AICc values (Burnham and Anderson, 2002) as the fit statistic to determine the optimal number of clusters for the element data set within the current study (i.e., we set up a new classification for the current sample rather than using element type classification from a previous study of red-backed fairy-wrens, Schwabl et al., 2015). This yielded 25 element types, which were then visually inspected to confirm that they were classified appropriately.

In-nest call elements were classified visually using the element comparison interface in Luscinia (Lachlan, 2015). This yielded 25 element types per individual, which is similar to the number of element types per song for this species (Schwabl et al., 2015, current study). Since in-nest calls typically have a smaller number of elements overall than songs (**Figure 1**), 25 representative elements for each female were chosen from a sample of five in-nest calls.

This generated a sample of representative exemplar elements, 175 maternal in-nest call elements (7 mothers × 25 elements each) and 200 offspring song elements (8 offspring × 25 elements each) for the comparison between maternal in-nest call elements and their offspring's adult song elements. For the comparison between maternal song elements and their offspring's adult song elements, we used a sample of 125 maternal song elements (5 mothers whose offspring's adult songs were also recorded × 25 elements each) and 125 offspring song elements (5 offspring whose mother's full songs were also recorded × 25 elements each). For the comparison between maternal song elements and maternal in-nest call elements, 125 maternal in-nest call elements (5 mothers × 25 elements each) were compared with 125 full song elements (5 mothers × 25 elements each) from those same mothers.

In each separate comparison (maternal in-nest call vs. offspring adult songs, maternal songs vs. offspring adult songs, and maternal songs vs. maternal in-nest calls), we made pair-wise comparisons of every element in the sample (i.e., each maternal element was compared to each offspring element in the sample, not just her own offspring). We used the dynamic time-warping

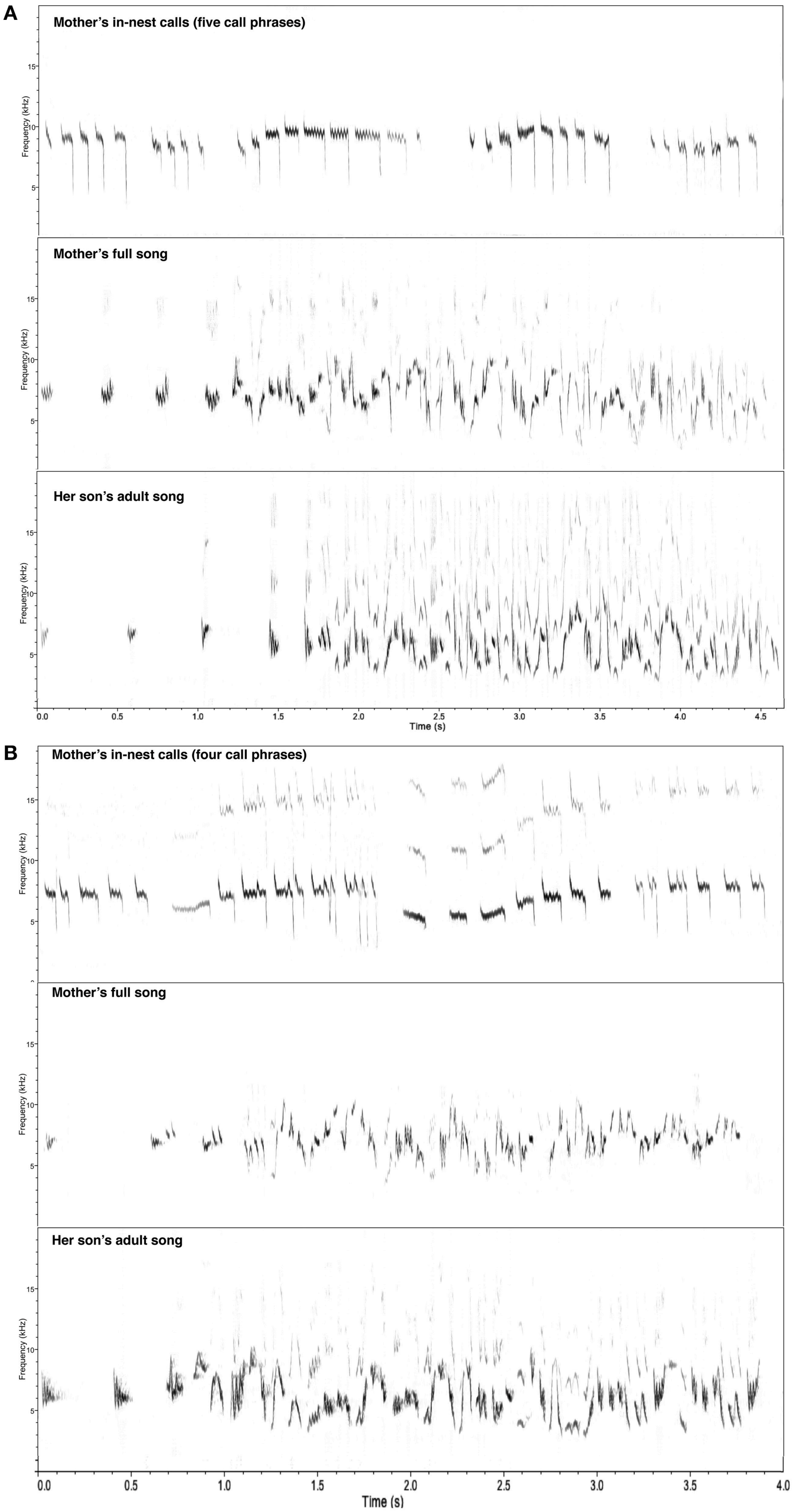

FIGURE 1 | Example spectrograms of female in nest calls (top panels), her full songs (center panels), and her son's adult songs (bottom panels). (A) First mother-son pair example from our population. **(B)** Second mother-son pair example from our population.

(DTW) algorithm in the software package Luscinia (Lachlan, 2015). This algorithm searches for an optimal alignment between two time series on the basis of the Euclidean distance between acoustic features. This method is more appropriate, in this context, than other automated measurements of song similarity (e.g., spectrographic cross-correlation, Clark et al., 1987), because we do not expect offspring to produce near-exact copies of tutor's song elements, but rather to produce variations of tutor elements that have similar, but modified shape and may differ in frequency and time characteristics.

In our analysis, the acoustic features we used were spectrograph measures calculated from tracing a contour over each element, and included: time, relative position, peak frequency, mean frequency, median frequency, peak frequency change, mean frequency change, median frequency change, and frequency bandwidth (additional parameter settings in Luscinia: compression factor: 0.001, minimum element length: 10, SD ratio: 0.5, maximum warp 60%, cost for stitching syllables: 1, cost for alignment error: 1, syllable comparison by individual element, with frequencies log transformed). In Luscinia (Lachlan, 2015), frequency characteristics are measured by analyzing many spectral slices of the signal, and the actual measurement of each slice's frequency is made from a power spectrum, rather than from the original spectrogram.

We checked that DTW similarity assignments matched the subjective assessments of human observers using the element dendrogram visual display in Luscinia. The output of the DTW analysis is a dissimilarity score between each pair of elements in our sample. Therefore, we created three matrices of dissimilarity comparing: (1) maternal in-nest call elements and offspring's adult song elements ($n = 8$), (2) maternal song elements and offspring's adult song elements ($n = 5$), and (3) maternal song elements and maternal in-nest call elements ($n = 5$).

Each matrix of dissimilarity was analyzed separately using principal coordinate analysis (PCoA) with the R-package software for Multivariate and Spatial analysis version 4 (Casgrain and Legendre, 2001). PCoA is a data reduction and ordination technique commonly used for cluster analysis (Legendre and Legendre, 1998) that ordinates distances between objects (in this study, elements) in multi-dimensional space, while preserving Euclidean distances between those elements. It is conceptually similar to principal components analysis (PCA); however, PCoA conserves distances generated from any (dis)similarity value allowing a more flexible analysis of the complexity of the data. In addition, the orthogonal nature of PCoA has the advantage of satisfying assumptions of independence when testing statistical hypotheses.

From the matrix of dissimilarity, PCoA creates a set of independent axes that position the objects (in this study, elements) in reduced space based on their similarity between each other (Legendre and Legendre, 1998), where objects ordinated closer to one another are more similar than those ordinated further away. Each axis has an eigenvalue whose magnitude indicates the amount of variation captured in that axis and the first five eigenvalues explain most of the variation in the data set (Baker and Logue, 2003). The first five PCoA coordinate values represent our five similarity scores per element, which we then averaged to create an average similarity score. In this analysis, smaller scores represented higher similarity. For more details on the methods see Colombelli-Négrel et al. (2012) and Colombelli-Négrel et al. (2016).

We then performed statistical analyses with SPSS 18.0 for Windows (SPSS Inc., Chicago, USA). We used the five similarity scores and the average score in a MANOVA and then in a discriminant function analysis (DFA) to test for acoustical similarity in elements between individuals. The percentage of correct classification by chance for the DFA was calculated by dividing 100% by the number of individuals used in each analysis. To determine if offpring song elements were more similar to maternal in-nest call elements or maternal song elements, we compared the average similarity scores per individual using a paired *t*-test. We tested for the relationship between offspring's element similarity to their mother's in-nest call elements and to their mother's song elements using a linear regression.

Ethics Approval Statement

All work was conducted with approval from appropriate animal ethics and permitting agencies (Cornell University Animal Care and Use Committee approval 2009-0105, James Cook University Ethics approval number A1340, and Queensland Government Environmental Protection Agency Scientific Purposes Permit number WISP07773610).

RESULTS

Similarity between Maternal In-Nest Calls and Offspring Adult Songs

The first eigenvalues extracted by PCoA accounted for 74.72% of the variance in the data (with 93.15% accounted for by the first five eigenvalues). We found that the element pair comparison (maternal in-nest call vs. offspring song elements) differed significantly between individuals for one of the five similarity scores and for the average score (**Table 1A**), suggesting that the song elements of each offspring were more similar to the in-nest call elements of their own mother than to the elements of any other female (see also **Figure 2**). Cross-validated DFA classified 24.5% of elements to the correct mother-offspring pair ($n = 8$), which was higher than the percentage of correct classification by chance (12.5%).

Similarity between Maternal Songs and Offspring Adult Songs

The first eigenvalues extracted by PCoA accounted for 76.75% of the variance in the data (with 94.72% accounted for by the first five eigenvalues). We found that the element pair comparison (maternal song elements vs. offspring song elements) did not differ significantly between individuals for five similarity scores but differed for the average score (**Table 1B**; see also **Figure 2**), again suggesting that the average song elements of each offspring were more similar to the song elements of their own mother than to the elements of any other female. Cross-validated DFA classified 32% of elements to the correct mother-offspring pair ($n = 5$), which was higher than the percentage of correct classification by chance (20%).

TABLE 1 | Results of dynamic time warping comparison and principal coordinate analysis (PCoA).

Dependent variable	*df*	*f*	*p*	Eta^2
(A) OFFSPRING SONG VS. MATERNAL IN-NEST CALL ELEMENTS ($n = 8$)				
Score 1	7	2.14	0.04	0.80
Score 2	7	1.16	0.33	0.49
Score 3	7	1.00	0.43	0.42
Score 4	7	0.39	0.90	0.17
Score 5	7	0.63	0.73	0.27
Average	7	2.25	0.03	0.83
(B) OFFSPRING SONG VS. MATERNAL SONG ELEMENTS ($n = 5$)				
Score 1	4	1.78	0.14	0.53
Score 2	4	0.75	0.56	0.23
Score 3	4	1.26	0.29	0.38
Score 4	4	1.27	0.29	0.39
Score 5	4	1.82	0.13	0.54
Average	4	3.22	0.01	0.82
(C) MATERNAL SONG VS. IN-NEST CALL ELEMENTS ($n = 5$)				
Score 1	4	3.47	0.01	0.85
Score 2	4	0.62	0.65	0.20
Score 3	4	407.20	<0.0001	1.00
Score 4	4	51.83	<0.0001	1.00
Score 5	4	7.42	<0.0001	1.00
Average	4	5.21	0.001	0.96

We calculated five element similarity scores for the structural similarity between each pair comparison, which we then averaged to create our average similarity score. Eta^2 is presented as a measure of effect size for each similarity score.

Offspring Song Similarity to Maternal In-Nest Calls vs. Maternal Songs

We next determined if adult songs of offspring were more similar to the maternal vocalizations they heard as embryos (in-nest calls) or maternal songs (mother's full songs). Comparisons of the averaged similarity scores per individual showed that offspring song elements were more similar to maternal song elements than maternal in-nest call elements (Paired t-test: $df = 4$; $t = 20.376$; $p < 0.0001$; **Figure 2**).

Similarity between Maternal Songs and Maternal In-Nest Calls

The first eigenvalues extracted by PCoA accounted for 53.89% of the variance in the data (with 86.27% accounted for by the first five eigenvalues). We found that female song elements were more similar to their own in-nest call elements than to the elements of any other female: the element pair comparison (maternal in-nest call vs. maternal song elements) differed significantly between individuals for four of the five similarity scores and for the average score (**Table 1C**). Cross-validated DFA classified 79.2% of elements to the correct female ($n = 5$), which was higher than the percentage of correct classification by chance (20%). In addition, offspring that produced song elements highly similar to their mother's song elements also had song elements highly similar to their mother's in-nest call elements (Linear regression: $t = 6.277$, $\beta = 0.964$, $p = 0.008$; **Figure 3**).

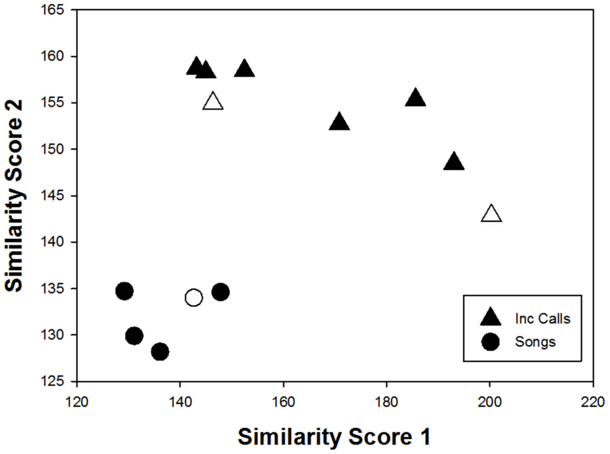

FIGURE 2 | Scatterplot of similarity score 1 vs. 2 showing the distribution in space of offspring song elements (averaged per individual) in relation to their maternal in-nest call elements (triangles; $n = 8$) and maternal song elements (circles; $n = 5$): offspring song elements were more similar to maternal song elements than maternal in-nest call elements (smaller scores on z and y represented higher similarity). The black circles and triangles represent the male offspring and white circles and triangles represent the female offspring.

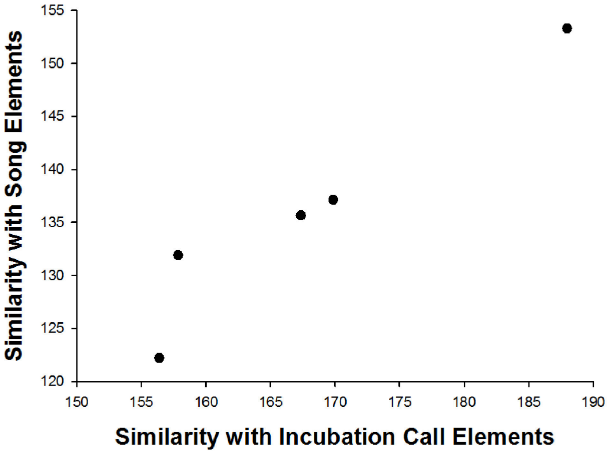

FIGURE 3 | A positive correlation between offspring element similarity to their mothers' in-nest call elements and to their mothers' song elements: offspring that produced song elements highly similar to their mothers' song elements also had song elements highly similar to their mothers' in-nest call elements. Elements with the smallest similarity values were more similar to their mothers' elements than those with highest values.

DISCUSSION

We found that adult birds produced song elements similar to the maternal in-nest call elements they heard as embryos in the nest as well as to their mother's full songs. However, offspring song elements were more similar to their mother's song elements than to the maternal in-nest call elements. Though we do not rule out genetic inheritance of song traits in our study, cultural transmission is extremely well supported across songbirds and likely also occurs in our study species (Slater, 1986; Podos et al., 2004). As such, our results, combined with previous studies of vocal kin recognition in red-backed fairy-wrens (Colombelli-Négrel et al., 2016) and embryonic vocal learning in superb fairy-wrens (Colombelli-Négrel et al., 2012, 2014), provide evidence that red-backed fairy-wrens incorporated both types of maternal elements into their crystallized songs, where they could serve as kin signatures. These elements were likely learned from their mothers throughout development, but perhaps also from within the egg. These signatures could be used for kin recognition, and as such, may increase the survival and reproduction of both sender and receiver. We also found that offspring that produced song elements highly similar to their mother's song elements had song elements highly similar to their mother's in-nest call elements, which suggests there is variation between young birds in how well they learn to produce elements similar to their maternal tutor in both contexts.

We hypothesized that if red-backed fairy-wrens use song to recognize kin, then the kin signatures individuals used as nestlings may be preserved in their adult songs, as this would facilitate kin recognition. Our result that offspring adult songs are similar to their mother's in-nest calls provides some indirect support for this hypothesis. However, it should be noted that the DFA assigned only 24.5% (in-nest calls) and 32% (songs) of elements to the correct mother-offspring pair, suggesting some overlap between individuals. These results may be explained by low variation between the individuals (possibly due to low sample size) or because there is some natural vocal variation within individuals over time (see Ellis, 2008). For example, Matrosova et al. (2009, 2010) showed that alarm call structures were unstable over time. In our case, a young bird's song may differ from its mother's vocalizations because it has changed over time, even though the bird learned the song from its mother originally. This may be expected if the vocalization converges on a group signature (Radford, 2005), if it encodes local dialect (Nelson and Poesel, 2007), and/or if other information influences vocalization structure (such as dominance status, Mathevon et al., 2010). Another potential explanation could be that young bird's songs differ from their mothers' because they learn vocal elements from multiple tutors. They may learn from their social father, which has been shown in several closely related species (Greig et al., 2012; Evans and Kleindorfer, 2016), but also possibly from auxiliary helpers and other nearby adults.

It is also important to note that further testing is required to confirm that this species is indeed using vocal kin signatures to recognize genetic relatives (e.g., with playback experiments, sensu Akcay et al., 2013). In addition, since offspring songs were more similar to maternal full songs, and there is high similarity between a mother's in-nest calls and her full songs, the similarity between offspring adult songs and maternal in-nest calls may only emerge due to multicollinearity. In other words, offspring may learn their adult song elements only from the songs of tutors they hear late in development (e.g., their mother's full songs), and not from maternal in-nest calls, meaning the similarity between offspring adult songs and maternal in-nest calls may exist only due to similarity between maternal in-nest calls and maternal full songs.

An alternative interpretation is that offspring learn the elements in their full songs from both their mother's in-nest calls and their mother's full songs, which would suggest that song learning occurs both within the egg/soon after hatch and later in development. It was discovered very recently that birds can discriminate between individual's calls and songs as embryos (Colombelli-Négrel et al., 2014; Colombelli-Négrel and Kleindorfer, unpublished data). Our results may provide further evidence to suggest that embryonic chicks may not only hear and respond to vocalizations while in the egg, but also may retain elements heard prenatally from those vocalizations (Colombelli-Négrel et al., 2012, 2014, 2016), and produce them later in life. Although future studies are needed (e.g., cross-fostering experiments that test whether embryos learn acoustic elements, controlling for genetic inheritance), our results may shed light on how birds acquire elements in their crystallized songs, suggesting that in some species, they may be acquired at an earlier developmental stage than previously believed. This illustrates a need for further investigation of the timing of avian vocal development, since classic and widely accepted models of song learning in birds suggest that the sensitive period for song acquisition begins much later, and propose that exposure to songs before 10 days post-hatch has no effect on learning (Marler, 1970).

Timing and length of the sensitive period for song acquisition vary widely across bird species (Marler, 1991; Brainard and Doupe, 2002). Both appear to be flexible, and tend to coincide with social and environmental contexts that are most ideal for appropriate song acquisition (Kroodsma and Pickert, 1980; Slater et al., 1992). So why would the sensitive period extend to such an early developmental stage in some species? For red-backed and other fairy-wrens (*Maluridae)*, the sensitive period may include an extraordinarily early developmental stage because these species may experience particularly strong selective pressure for accurate kin signature acquisition. This could be due to the critical importance of accurate kin recognition in a nest provisioning context, since there is a strong threat of brood parasitism from many cuckoo species (*Cuculinae* spp.; Langmore et al., 2003; Colombelli-Négrel et al., 2012), and in a cooperative breeding context, since auxiliary helpers must ensure they assist adult kin (i.e., parents) and care only for siblings that are genetically related (McDonald and Wright, 2011; McDonald, 2012).

Our study helps to address how kin signatures are acquired within an individual's lifetime. Our results suggest that kin signatures are acquired from the mother (though may additionally be acquired from other adults present, including the social father and helpers, Greig et al., 2012) with a potential role of both prenatal and post-hatching learning. When senders benefit from being recognized, selection is expected to act directly on their signal properties to enhance the reliability of their kin signatures (Beecher, 1988). Early developmental learning of kin signatures could benefit both individuals that produce vocal kin signatures and those that recognize and respond to them, by minimizing mistakes in signature acquisition through limiting possible tutors to those that sing frequently within close range of the nest.

CONCLUSIONS

Currently, our understanding of vocal kin recognition systems remains incomplete (Rendall et al., 1996; Knörnschild et al., 2012; Akcay et al., 2013). General explorations of whether genetic relatives share vocal kin signatures, such as the present study, are needed across taxa to understand the occurrence and extent of vocal kin recognition. In addition, studies like ours help to further our understanding of how vocal kin recognition systems may evolve, by helping to elucidate developmental timing of kin signature acquisition, and general adaptive benefits of vocal kin recognition systems for maximizing indirect fitness benefits and minimizing costs of inbreeding. Gaining a more comprehensive understanding of kin recognition signaling systems, including the developmental and evolutionary mechanisms that produce them, helps us to better understand how adaptive signaling systems like these come to be, both within the span of an individual's lifetime and over evolutionary time.

AUTHOR CONTRIBUTIONS

JD, MW, and DC all contributed to the conception and design of the work. JD and DC both contributed to the acquisition, analysis, and interpretation of data for the paper. JD drafted the paper. JD, MW, and DC all contributed to revising the paper critically for important intellectual content.

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Mismatch in sexual dimorphism of developing song and song control system in blue-capped cordon-bleus, a songbird species with singing females and males

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Brain song control regions of adult passerine birds are sexually dimorphic in species such as the zebra finch (*Taeniopygia guttata*) in which males sing whereas females do not. In many tropical bird species, however, females sing as well. Here we study for the first time the ontogeny of the song control system and the song in a species in which both male and female sing regularly. In blue-capped cordon-bleus (*Uraeginthus cyanocephalus*), a distant relative of the zebra finch, both males and females start singing at around 30–40 day post-hatching (dph). First we quantified that sex-specific differences in song features emerged only in adulthood, after 250 dph of age: Adult females sang complex songs, which were slightly shorter and contained fewer syllables as compared to the males. Second, the development of forebrain song control regions HVC (proper name) and RA (nucleus robustus arcopallii) of blue-capped cordon-bleus was quantified in both sexes at 20, 30, 50, 100, 150, 250 dph as well as in old adults. The volume and neuron numbers of the HVC and RA were sexually dimorphic throughout the entire development and remained sexually dimorphic in adulthood. Since singing developed in a non sex-specific way until 250 dph, neural sex differences to a large extent precede the behaviorally (song) sex differences. This suggests that these neuroanatomical sex differences are not causally related to the sexual differentiation of song patterns in this species.

Keywords: song system, song development, female songbird, male songbird, sexual dimorphism

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INTRODUCTION

A frequently used argument to suggest that female songbirds are not capable of singing or sing only simple songs is that the neuroanatomy of the forebrain song control areas (collectively called the song system) is sexually dimorphic (Nottebohm and Arnold, 1976; DeVoogd and Nottebohm, 1981; Brenowitz and Arnold, 1986; DeVoogd et al., 1993; Gahr et al., 1998; MacDougall-Shackelton and Ball, 1999). In particular, song control areas such as HVC (letter based name) and RA (nucleus robustus archopallii) are much smaller and contain much fewer neurons in the adult female than in the adult males of all songbird species studied so far (Gurney, 1981; Konishi and Akutagawa, 1985; Gahr et al., 1998, 2008; Jawor and MacDougall-Shackleton, 2008; Schwabl et al., 2015). The HVC of the descending song production pathway of the song system is a sensorimotor integration area implicated in controlling the temporal dynamics of adult song and the motor-cortical RA controls the temporal-spectral details of the sounds (Yu and Margoliash, 1996; Hahnloser et al., 2002; Amador et al., 2013). The prime example of this sexual dimorphism of the song control system and singing is the zebra finch, in which stark neuroanatomical sex differences correlate with the lack of singing of adult females (Nottebohm and Arnold, 1976), even after testosterone treatment, a paradigm that induces singing in males of all species tested (for review Gahr, 2014). This neuroanatomical sex-difference of zebra finches emerges during ontogeny due to genetic and endocrine mechanisms (Gurney and Konishi, 1980; Wade and Arnold, 1996; Gahr and Metzdorf, 1999; Holloway and Clayton, 2001; Agate et al., 2003). In particular, the strong increase in sex differences of song control areas around the time when first song precursors are uttered by male zebra finches is thought to reflect the song development in males and the lack of song capacity of females (Konishi and Akutagawa, 1985). Likewise, in the canary (*Serinus canaria*), the other songbird species in which the development of the song system has been studied in detail, the neuroanatomy of the song system diverges strongly between males and females during ontogeny (Gahr et al., 1997). In adulthood, female canaries sing only occasionally and if at all, generally with few unstructured songs compared to adult males (Pesch and Güttinger, 1985). Female canary song can be activated by testosterone (e.g., Shoemaker, 1939; Hartley and Suthers, 1990; Hartog et al., 2009). This potential to sing correlates with the differentiation of their song control areas, which is relatively smaller as compared to canary males but relatively larger as compared to female zebra finches (Nottebohm and Arnold, 1976; Nottebohm, 1980).

However, these observations of sexually dimorphic song system differentiation are in strong contrast with the report that female singing is the rule but not the exception among songbird species (for reviews: Slater and Mann, 2004; Riebel et al., 2005; Garamszegi et al., 2006; Gahr, 2014; Odom et al., 2014). Previously, it was thought that females of only some species sing (e.g., Beletsky, 1983; Gahr and Güttinger, 1985; Hoelzel, 1986; Langmore et al., 1996; Yamaguchi, 2001). One reason for this discrepancy might be that song system development was never studied in a species in which females sing regularly. To this end, we studied both the song system development and the song development in a songbird species, the blue-capped cordon-blue (*Uraeginthus cyanocephalus*) in which males and females sing frequently both during ontogeny and in adulthood (Geberzahn and Gahr, 2011, 2013). Previous studies showed that male and female cordon-bleus learn parts of their song from both adult male and female tutors (Geberzahn and Gahr, 2013), with the adult female song being slightly shorter and less diversified than the male song (Geberzahn and Gahr, 2011). Here we quantify the song development of female and male cordon-bleus from juvenile age until adulthood, focusing on song parameters that were indicated previously to differ between adult males and females. In zebra finches song development ends with reproductive maturation at around 100 days post-hatching (dph) (Immelmann, 1969). Since reproductive activities of cordon-bleus start around 6–8 month (i.e., about 250 dph) we analyzed the song development till 250 dph (young adults) in detail. This was paralleled with the study of the developing song system neuroanatomy, in particular the volume of HVC and RA as well as their respective neuron numbers. Thus, our integrative study of the song system and song development of male and female cordon bleus should inform about the interrelationship between the neural and the behaviorally song phenotype during ontogeny.

MATERIALS AND METHODS

Animals

Blue-capped cordon-bleus (*Uraeginthus cyanocephalus*) originated from the breeding colony of the Max-Planck Institute for Ornithology in Seewiesen, Germany. Birds were kept on a 14:10 light:dark schedule (lights on 07:00–21:00 Central European time) at 22°C and ca. 50% humidity and received water and food *ad libitum*. From nutritional independence onwards, birds were kept in small social groups in cages till 250 dph. Therefore, juveniles experienced a normal social environment between hatching and 250 dph. Afterwards, birds were moved to same sex aviaries until they were paired and given the opportunity to breed. Adults used in this study were paired, and in breeding condition at recording or when sacrificed for neuroanatomical analysis. These adults were about 4–5 years old (mean ± SD, minimum: males: 1642 ± 194, 1220 days; females: 1560 ± 221, 1395 days). All experimental procedures were conducted in accordance with German National Laws and approved by the Government of Upper Bavaria.

The study of song development was conducted with a cohort of 10 males and 10 females. Juveniles were recorded around 50, 100, 150, and 250 dph as well as at the age of about 4–5 years. The neuroanatomical study was conducted with a second cohort of blue-capped cordon-bleus. We included the following age-groups: 20 dph ($n = 6$ males and $n = 7$ females), 30 dph ($n = 5$ males and $n = 5$ females), 50 dph ($n = 5$ males and $n = 5$ females), 100 dph ($n = 5$ males and $n = 5$ females), 150 dph ($n = 4$ males and $n = 4$ females), 250 dph ($n = 3$ males and $n = 3$ females), adults ($n = 7$ males and $n = 7$ females). The HVC volume of one 20 dph female could not be analyzed due to damage of the brain surface.

Song Recording and Analysis

In order to obtain song that can be traced back unequivocally to a given subject, each bird was transferred temporarily to a sound-attenuating chamber where song recordings were made following procedures described in Geberzahn and Gahr (2011) using Sound Analysis Pro (SAP) software (Version 2.062) at a sampling rate of 44 kHz and 16-bit resolution (Tchernichovski et al., 2004; freely available at http://soundanalysispro.com). Thus, we recorded so-called undirected songs.

For analysis, we considered the first 50 song-like vocalizations that a subject produced after being moved to the recording chamber. Then, the following parameters, previously found to be sexually dimorphic in adult blue-capped cordon-bleus (Geberzahn and Gahr, 2011) were measured: the song duration, the syllable repertoire and the coefficient of variation (CV = SD/mean) of pitch goodness. The latter is described as the harmonicity of the sound and its CV indicates the degree of stereotypy of the harmonicity of a syllable. Songs were defined as sequence of at least three different syllables with pauses shorter than 600 ms. For each song, syllables were automatically delineated using a constant threshold for amplitude (23 dB) and entropy (–2.1) in the features batch window of SAP as described previously (Geberzahn and Gahr, 2011). In a next step, we printed images of spectral derivatives using SAP. Based on these images and the automatic delineation of syllables we visually compared song-like renditions of a given subject and labeled the same syllables with the same number according to their overall spectral-temporal appearance. This allowed us to determine the repertoire size of syllable types for each subject. Blue-capped cordon-bleus usually start a song with a variable number of introductory syllables, which were labeled as introductory syllables and not further categorized. We excluded introductory syllables from all further analysis.

The features batch procedure of SAP provided us with parameter tables for all syllables and we manually added a column with the “syllable repertoire” that resulted from visual inspection (see above). From these parameter tables we extracted the pitch goodness (mean, SD and CV). We calculated the coefficient of variation (CV = SD/mean) of pitch goodness of the four most frequent syllables similar to previous works (Geberzahn and Gahr, 2011). For those syllables we selected the first 20 renditions that occurred in the analyzed songs. For between-subjects comparisons, we chose the CV for the syllable with the lowest CV for each subject, i.e., the syllable with the lowest variability from rendition to rendition (minimum CV of pitch goodness per subject).

Histology

When the birds of the neuroanatomical cohort reached the ages of interest for this study (see above), they were decapitated, brains were extracted, immediately frozen in dry ice and then stored at −80°C until sectioning. The frozen brains (right hemisphere only) were cut on a cryostat (JUNG CM 3000 LEICA) into 20 μm sagittal sections and mounted onto Fisher Superfrost Plus slides. Sections were mounted onto five different sets of slides so that adjacent sections could be analyzed by different methods. After drying overnight, one series of sections (i.e., every fifth slide) was rehydrated, Nissl-stained with 0.1% Thionin (Sigma), dehydrated, immersed in xylene and cover-slipped with Roti-histokit II mounting medium. The other series of sections were stored at −80°C until use. We performed the measurements of the Nissl-stained HVC and RA areas (unilateral), delineated based on their cytoarchitecture, using the built-in cursor and measurement tools in Image J software (NHI, National Health Institute, USA). All brains were coded so that the delineations made by the observers were blind to the sex and age of the sections they measured. The RA and HVC volume, respectively, was calculated by summing the areas, multiplied by section thickness, and multiplied by 5 (sampling interval).

Immunohistochemistry

In order to estimate the number of neurons in HVC and RA of 20, 100 dph, and adult males and females, we performed immunohistochemistry with antibodies against the neuron-specific RNA-binding protein HuC/D (mouse monoclonal, clone 16A11; Molecular Probes, Eugene, OR). This antibody has previously been successfully used to label neurons in a broad range of species including songbirds (e.g., Marusich et al., 1994; Barami et al., 1995; Vellema et al., 2010), and has been shown to recognize a 40-kDa band on Western blots of extracts of human neurons, as well as mouse and avian brains (Marusich et al., 1994).

We immunostained one series of slides of each brain, i.e., every fifth section. After defrosting and drying, sections were fixed for 40 min in a 4% formaldehyde solution and then washed one time in phosphate-buffered saline (PBS) and three times in PBS containing 0.1% Triton X-100 (PBT) for 5 min each on a rotating shaker. Following that, the sections were incubated overnight at room temperature in PBT, containing 0.02% NaN_3, 10% horse serum (AbD Serotec, Oxford, UK), and primary antibodies against HUC/D (2.5 μg/ml). After three washes in PBT, sections were incubated in Alexa Fluor 555-conjugated donkey anti-mouse secondary antibodies (1:500; Molecular probes) for 1 h at room temperature. After another two PBT rinses, brain sections were DAPI stained for 5 min, washed in PBT, and embedded in Vectashield (Vector Laboratories, Burlingame, CA) to prevent photo bleaching and cover slipped. The slides were stored horizontally at 4°C until microscope analysis.

Neuron Quantification

Brain sections were examined with a Leica DM6000B digital fluorescence microscope (Leica Microsystems) equipped with a Leica DFC420 5 megapixel CCD digital color camera. DAPI and Cy3 filter cubes were used to visualize DAPI and the Alexa-555 antibody, respectively. The resulting photomicrographs were merged and adjusted for color, brightness and contrast with Photoshop CS2 (Adobe Systems Inc., San Jose, CA) to create multicolor images.

The number of Hu-positive cells in HVC and RA were manually counted under the microscope in three randomly selected brain sections from each bird. A counting grid was projected onto HVC and RA, and three random counting frames of 0.01 μm^2 were chosen for each brain section to estimate the neuron density in the areas of interest. The Hu-positive cell counts were performed using a 20× magnification and cells were included if the nuclei of Hu-labeled cells were located inside the counting frame, including cells that traverse the upper and right border, but excluding the cells that traverse the lower and left border of the counting frame. Additionally, to minimize cell count overestimation due to split cells on the counting plane, only cells of which the entire nucleus could be brought in focus within the 20-μm z-stack were included in the counts. Total neuron numbers for HVC and RA were obtained by multiplying the neuron density estimates with the obtained volume measurements from the Nissl-stains.

Statistical Analyses

We performed all statistical analyses with the JMP v. 10.0 software (2012, SAS Institute Inc.). To examine differences between song control regions (HVC and RA) across age- and sex-classes we applied ANOVAs with age and sex as independent variables using a general Linear Model procedure in separate data sets for HVC volume, HVC neuron numbers, RA volume and RA neuron numbers. RA data were log transformed. For song development, we used a "split plot design" as the basis for a "repeated measures across time design" to test for the differences across time and between sexes. For this, song duration, syllables repertoire and coefficient of variation of pitch goodness were used as dependent variables (log transformed). The effect of sex (male or female) is tested with respect to the variation from bird to bird. The within subject effect ("age" effect) is tested with respect to the variation from age to age of the same bird. The within bird variability—reflected in the residual error captured with sex nested within bird [bird (sex)]—was an added effect to the model and assigned as random attribute. For the neuroanatomical development and the song development, after an overall F-test showed significance, we used a Tukey's *post-hoc* test with $\alpha = 0.05$ to evaluate differences between specific means. Data are given as mean ± SEM.

RESULTS

Song Development

Juvenile males and females started to utter their first song-like vocalizations, so-called sub-songs around 30–40 dph (males: 32 ± 4 dph; females 31 ± 5 dph). All animals, males and females, developed the typical song of blue-capped cordon-bleus with an increased pitch of syllables that are uttered toward the end of the song (**Figures 1A,F**; Geberzahn and Gahr, 2011). This is best seen when comparing the pitch of the first and the last syllable of a given song and was already obvious at 50 dph in some songs (**Figure 1J**). In the following we focus on song duration, syllable repertoires and the CV of pitch goodness. As detailed below, in general, male and female songs were similar during development, even when they differed in adults.

We found a significant effect of age on song duration [$F_{(4,\ 64.65)} = 9.998$, $p < 0.0001$]. However, song duration of blue-capped cordon-bleus was sexually dimorphic only in adults (**Figures 1**, **2A**; males 2497 ± 147 ms; females 1750 ± 175 ms; *post-hoc*: $p = 0.0034$). This sexual dimorphism emerged due to the reduction in song duration in females during late ontogeny resp. early adulthood (i.e., after 250 dph) (*post-hoc* 250 dph vs. adulthood: $p = 0.0076$) while song duration of males did not change significantly after 250 dph (*post-hoc* 250 dph vs. adulthood: $p = 0.5591$). Song duration of 100 dph females (2842 ± 266 ms) did not differ from that of 250 dph females but differed significantly from that of adult females (*post-hoc*: $p = 0.0002$) (**Figure 2A**). This was not the case for 100 dph males (*post-hoc* 100 dph vs. adulthood; $p = 0.07$) (**Figure 2A**).

Cumulative curves of new syllable types plotted against the number of syllables analyzed were visually checked (e.g., Leitner et al., 2001; Geberzahn and Hultsch, 2003). The curves only reached an asymptote in adults, 250 and 150 dph birds, indicating that the amount of songs sampled was sufficient to cover the syllable repertoire. At 100 dph, the curve reached only saturation if we omitted the 15% of syllables that were not uttered a second time in the analyzed songs. Thus, the repertoire of 100 day old males and females might be higher than that reported in **Figure 2B** (see also **Figure 1**). At 50 dph, the syllable repertoire was not measurable due to the fact that the syllables were very variable and could not be assigned to syllable types (**Figures 1E,J**). Since the analysis of additional singing events of the 50-day birds did still not allow us assigning syllables to syllable types, we did not further pursue the analysis of the repertoire at this age.

For syllable repertoire, we found an effect of age [$F_{(3,\ 42.15)} = 82.25$, $p < 0.0001$] and an interaction between sex and age [$F_{(3,\ 42.15)} = 5.65$, $p = 0.0024$]. In males, syllable repertoire size dropped significantly from 100 to 150 dph (*post-hoc*: $p = 0.0452$), from 150 to 250 dph (*post-hoc*: $p = 0.0003$) but not between 250 dph and adulthood (*post-hoc*: $p = 0.08$) (**Figures 1**, **2B**). In females, syllable repertoires decreased significantly between 150 and 250 dph, and between 250 dph and adulthood (both *post-hoc* tests: $p < 0.001$). Syllable repertoire sizes differed significantly between males and females only at adulthood (males = 18.1 ± 1.4 syllables; females = 10.9 ± 1.3 syllables; *post-hoc* $p = 0.001$) (**Figures 2B**, **1**). This was due to a stronger reduction of syllable numbers in females as compared to males, which might be related to the sex-specific song duration of adults.

Despite the reduction of overt syllables of females after 250 dph, both sexes integrated considerable numbers of new syllables (i.e., syllables not uttered before) in their repertoire between 250 dph (**Figures 1B,G**) and adulthood (**Figures 1A,F**). Thus, blue-capped cordon-bleus have a very long sensorimotor learning period, much longer than that of zebra finches that last about till 120 after hatching (Immelmann, 1969). In addition, since our birds were moved to aviaries after 250 dph and thus exposed to conspecifics not heard before, cordon-bleus might have a much longer sensory song learning period than zebra finches that last about till 70 after hatching (Immelmann, 1969). Since this extended period of song learning and plasticity was unexpected, song development of young adult cordon-bleus (after 250 dph) was not studied in detail (this study and Geberzahn and Gahr, 2013) and we do not know yet till which age cordon-bleus are able to modify their songs.

We measured the CV of pitch goodness only of 150, 250 dph, and adult males and females, due to the problem of determining the syllable repertoires of earlier ages (50 and 100 dph, see above). We found an effect of age on the CV of pitch of goodness [$F_{(2,\ 25.86)} = 58.63$, $p < 0.0001$]. CV of pitch goodness decreased during ontogeny in both sexes i.e., syllables' harmonicity became more stereotyped with age but significantly only after 250 dph (*post-hoc* 250 dph vs. adulthood: $p < 0.0001$; **Figure 2C**). However, we neither found an effect of sex in the development

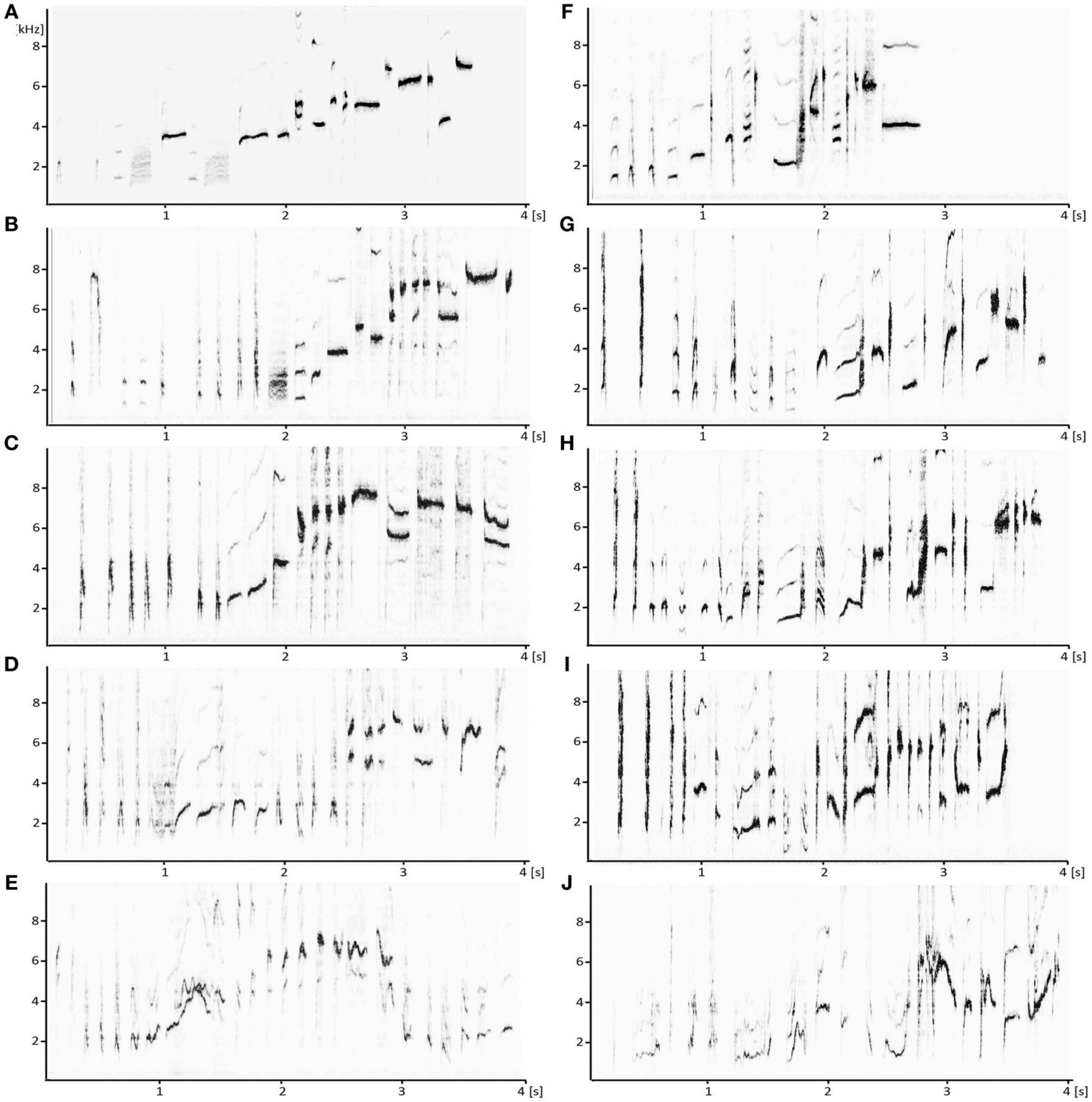

FIGURE 1 | Song development of a male (A–E) and a female (F–J) blue-capped cordon-bleu. Shown are sonograms of songs uttered at 50 **(E,J)**, 100 **(D,I)**, 150 **(C,H)**, 250 dph **(B,G)**, and in adulthood **(A,F)**. Note that songs of the female and male change considerably even after 250 dph, i.e., in adulthood.

of CV of pitch goodness [$F_{(1,\ 42.39)} = 0.0467$, $p = 0.83$] nor an interaction between sex and age [$F_{(2,\ 25.86)} = 0.6449$, $p = 0.53$]. Therefore, CV of pitch goodness did not differ significantly between sexes in difference to a previous study (Geberzahn and Gahr, 2011). The adult cordon-bleus of the present study were 4–5 years old and as such somewhat older than those studied previously (mean ± SD, minimum: females: 860 ± 226, 534 days; males: 915 ± 228, 578 days) (Geberzahn and Gahr, 2011). Thus, one possible explanation for this discrepancy between studies is that females change their harmonicity during adult life. Alternatively, stereotypy of harmonicity might strongly depend on the composition of the syllable repertoires between cohorts of cordon-bleus. In consequence of these contradictory results, we shall not further discuss potential sex differences in harmonicity.

In summary, song development (song duration, syllable repertoire size) of males and females was not sex-specific during most part of their ontogeny (till 250 dph), i.e., sex differences in song patterning emerged only during adulthood in this species. Reproductive activity starts around 6–8 month (i.e., about 250 dph) in cordon-bleus.

Development of the Volume of the Song Control Regions HVC and RA

HVC and RA volumes of males and females changed during development [HVC: $F_{(13,\ 56)} = 18.87, p < 0.0001$; RA: $F_{(13,\ 57)} = 24.37$, $p < 0.0001$; **Figures 3**, **4**, **5A,C**]. For both nuclei, there was a significant effect of age [HVC: $F_{(6,\ 56)} = 16.91, p < 0.001$; RA: $F_{(6,\ 57)} = 19.98$, $p < 0.0001$] and of sex [HVC: $F_{(1,\ 56)} =$

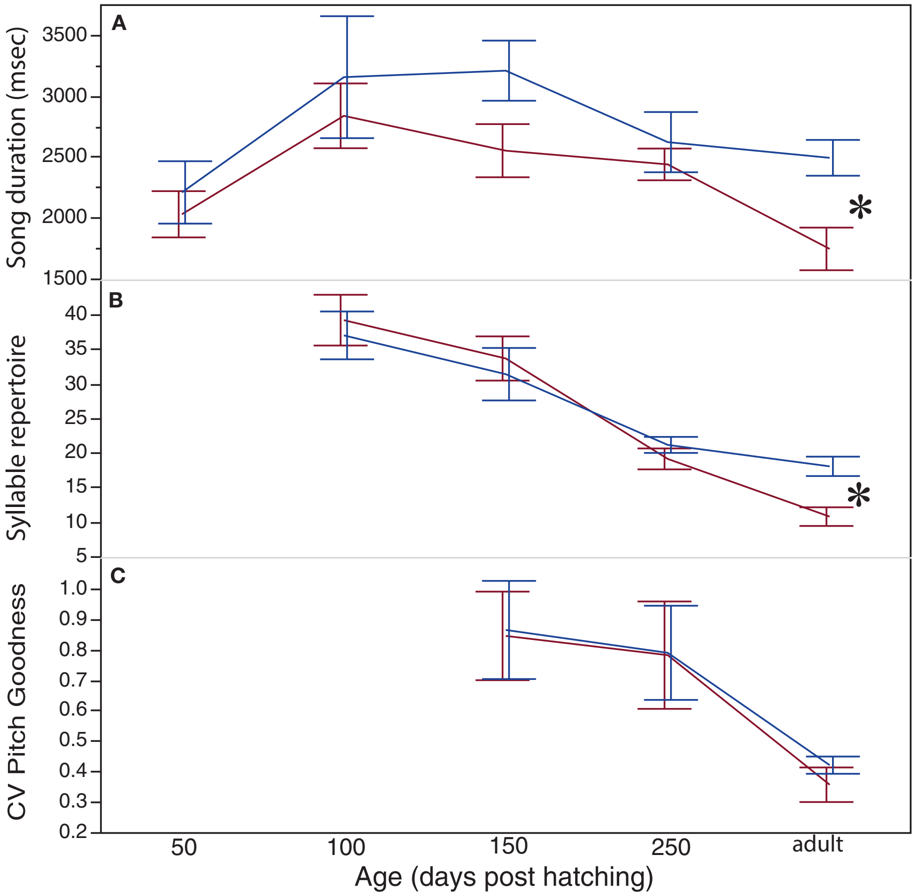

FIGURE 2 | Sexually dimorphic development of song pattern of blue-capped cordon-bleus. Depicted are the developments of song duration **(A)**, syllable repertoire size **(B)**, and of the harmonicity (CV of pitch goodness) **(C)**. Note that song duration and syllable repertoire size develop similar in males (blue lines) and in females (red lines) till 250 days of age and become sexually dimorphic (*$P < 0.05$) in adulthood. Data are presented as mean ± 1 SE.

16.20, $p = 0.0002$; RA: $F_{(1,\ 57)} = 14.83$, $p = 0.0003$]. Further, both nuclei changed similarly in males and females, i.e., there was no interaction between age and sex [HVC: $F_{(6,\ 56)} = 1.05$, $p = 0.4041$; RA: $F_{(6,\ 57)} = 3.09$; $p = 0.3744$]. In particular, HVC and RA volumes of males were significantly larger than those of females throughout all studied time-points (**Figures 5A,C**; **Tables 1A,B** for p-values of all *post-hoc* pairwise-comparisons between the sexes).

In males, HVC volumes of 20 dph birds were significantly smaller than those of all other age classes, except the 30 dph birds (**Figure 5A**; **Table 1A** for p-values of all *post-hoc* pairwise-comparisons between age-groups reported below). Further, from one studied developmental time-point to the next only the HVC size of 30 to 50 dph differed significantly. In females, HVC volumes of 20 dph were significantly smaller than those of 50, 100, and 150 dph birds but similar to those of 250 dph and adults. Only 100 dph females had significantly larger HVCs than adults. In summary, male HVC increased in volume till 50 dph and decreased again toward adulthood. In females, HVC increased in volume from 30 to 100 dph and decreased significantly afterwards so that HVCs of older (250 dph and

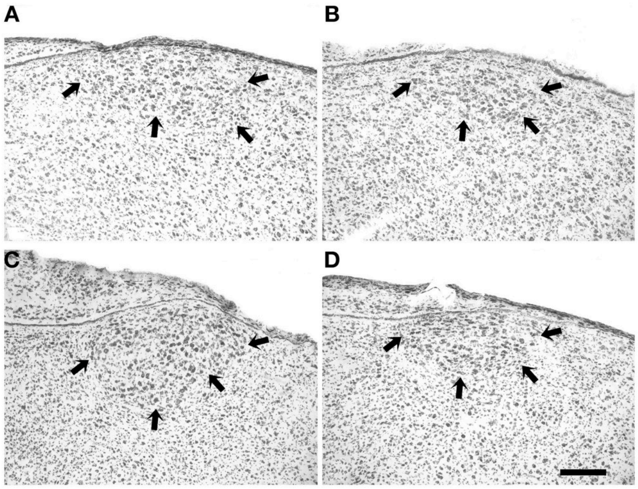

FIGURE 3 | Nissl-stainings of HVC (arrows) of a 20-day male (A), an adult male (C), a 20-day female (B), and an adult female (D) blue-capped cordon bleu. Scale bar is 100 µm.

adults) females were similar in size to those of very young females.

Likewise, RA volumes of males and females changed during development (**Figure 5C**; see **Table 1B** for *p*-values of all *post-hoc* pair-wise comparisons between age groups reported below). For the males, the RAs at 20 dph were significantly smaller than those of all older birds. From one developmental time-point to the next, the RA volume increased significantly from 20 to 30 and from 50 to 100 dph and decreased significantly from 100 to adulthood. In females, RA volumes increase significantly till 100 and 150 dph and then decreased till adulthood although this was only significant between 100 dph and adulthood. At the developmental peak of 100 dph, the RA volumes of males were significantly larger compared to all other time-points, the RA volumes of females were significantly larger than those of 20, 30, 50 dph and adult females.

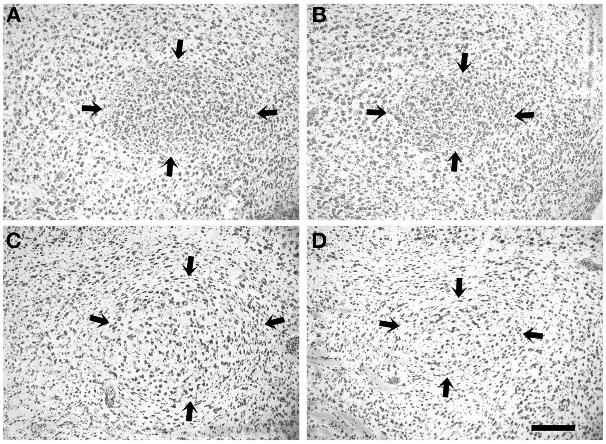

FIGURE 4 | Nissl-stainings of RA (arrows) of a 20-day male (A), an adult male (C), a 20-day female (B), and an adult female (D) blue-capped cordon bleu. Scale bar is 100 μm.

The degrees of sexual dimorphism (female to male ratio of mean volumes) of HVC and RA fluctuated between 0.7 and 0.5 during lifespan.

Development of HVC and RA Neuron Numbers

To further study the sexual dimorphism and development of HVC and RA we analyzed the neuron numbers of 20, 100 dph, and adult females and males (**Figures 5B,D**, **6**, **Table 2**). Neuron numbers of male and female HVCs [$F_{(5,\ 24)} = 34.54$, $p < 0.0001$] changed during ontogeny with an effect of age [$F_{(2,\ 24)} = 53.40$, $p < 0.0001$] and of sex [$F_{(1,\ 24)} = 14.53$, $p = 0.0008$] but no interaction between sex and age [$F_{(2,\ 24)} = 1.33$, $p = 0.28$] (**Figure 5B**, **Table 2A** for *p*-values of pair-wise comparisons

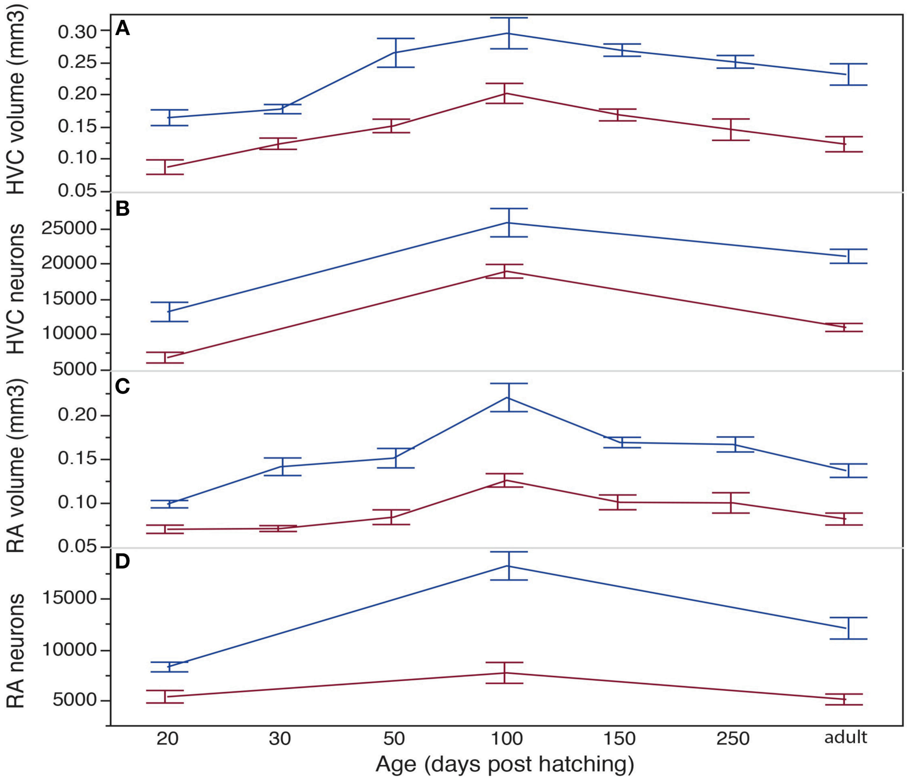

FIGURE 5 | The development of the HVC volume (A), HVC neuron numbers (B), RA volume (C), and RA neuron numbers (D) of female (red lines) and male (blue lines) blue-capped cordon-bleus. HVC and RA volumes and neuron numbers are sexually dimorphic throughout ontogeny and in adulthood. For the statistics of intra-sex comparisons see **Tables 1, 2**. Note the difference in scale between A and C, B and D. Data are presented as mean ± 1 SE.

TABLE 1 | Comparisons of HVC (A) and RA (B) volumes of females and males revealed by Tukey *post-hoc* tests.

Age	20 dph	30 dph	50 dph	100 dph	150 dph	250 dph	Adult
(A) HVC VOLUMES							
20 dph	**0.001**	0.325	**0.009**	**0.0001**	**0.001**	0.070	0.259
30 dph	0.997	<**0.0001**	0.679	**0.002**	0.218	0.923	1.00
50 dph	**0.003**	**0.017**	**0.002**	0.084	0.964	1.00	0.567
100 dph	**0.0001**	**0.001**	0.861	**0.012**	0.537	0.104	**0.001**
150 dph	**0.003**	**0.018**	1.00	0.943	<**0.0001**	0.924	0.145
250 dph	**0.043**	0.149	0.999	0.684	0.995	**0.005**	0.886
Adult	**0.049**	0.227	0.738	0.095	0.691	0.988	<**0.0001**
(B) RA VOLUMES							
20 dph	**0.001**	1.0	0.686	**0.0001**	**0.050**	0.107	0.749
30 dph	**0.0004**	<**0.0001**	0.846	**0.001**	0.111	0.195	0.900
50 dph	**0.0001**	0.987	**0.001**	**0.018**	0.703	0.810	1.00
100 dph	**0.0001**	**0.0001**	**0.003**	**0.001**	0.545	0.599	**0.005**
150 dph	**0.0001**	0.436	0.851	0.124	**0.001**	1.00	0.497
250 dph	**0.0001**	0.622	0.939	0.146	1.00	**0.01**	0.648
Adult	**0.005**	1.00	0.887	**0.0001**	0.196	0.365	<**0.0001**

P-values of female-female comparisons are in light-gray, those of male-male comparisons in dark-gray, and those of male-female comparisons are in the diagonal. Significant P-values are in bold. dph, age in days post-hatching.

between time-points reported below). In both sexes, neuron numbers increased significantly between 20 and 100 dph and dropped significantly between 100 dph and adulthood, so that adult HVC neuron numbers differed from those of 20 dph birds. Similar, in RA, neuron numbers changed during development [$F_{(5,\ 18)} = 21.74$, $p < 0.0001$] with an effect of age [$F_{(2,\ 18)} = 15.59$, $p = 0.0001$] and sex [$F_{(1,\ 18)} = 12.62$, $p = 0.0023$] but without interaction between age and sex [$F_{(2,18)} = 3.1$, $p = 0.07$] (**Figure 5D**, **Table 2B** for p-values of pair-wise comparisons between time-points reported below). Hence, the peak in neuron numbers at 100 dph of males and females was significant. RA neuron numbers dropped significantly after 100 dph till adulthood so that neuron numbers of adult females but not of adult males were similar to neuron numbers of 20 dph juveniles (**Figure 5D**, **Table 2B**). Further, neuron numbers of 20-, 100-day, and adult HVC and RA were significantly higher in males than females (**Figures 5B,D**; $p < 0.0025$ in all cases [**Tables 2A,B**]).

DISCUSSION

Sexually Dimorphic Development of HVC and RA does not Correlate with Sex-specific Song Development

Blue-capped cordon-blues are representative of many tropical and sub-tropical songbird species in which females sing regularly throughout life and sing a species-typical song pattern that is as well produced by their male conspecifics (for review: Odom et al., 2014). Thus, this species is a perfect model to study song and song system development of both males and females in contrast to the zebra finch, in which only the male is singing and in which song control areas are rudimentary in females (Nottebohm and Arnold, 1976). Nevertheless, volume and neuron numbers of forebrain song control regions HVC and RA that are crucial for song development and production are sexually dimorphic in adult blue-capped cordon-bleus, about 55 and 40% smaller, respectively, in females as compared to the males consisting of about 50% less neurons. Since, song patterns of adults (considering song duration and syllable repertoire size) are sexually dimorphic (this study; Geberzahn and Gahr, 2011), one might suggest a positive correlation between the neuroanatomy of the song system and sex differences in song. If this assumption were correct, blue-capped cordon-bleus would be similar to a group of species such as various wrens (e.g., Brenowitz et al., 1985; Brenowitz and Arnold, 1986; DeVoogd et al., 1988), Northern cardinals (*Cardinalis cardinalis*; Jawor and MacDougall-Shackleton, 2008; Yamaguchi, 1998), and starlings (*Sturnus vulgaris*; Bernard et al., 1993; Hausberger et al., 1995) in which small to medium sex differences in song pattern correlate with small to medium sex differences in the morphology of the song system. Sex-specific singing activity as the behavioral correlate of the observed neuroanatomical differences between male and female blue-capped cordon-bleus is unlikely since both sexes are able to sing equally frequently after removal of their mate (Geberzahn and Gahr, personal observation). Further, works in streak-backed oriol (*Icterus pustulatus)* showed that there is male-based sex difference in HVC morphology although females out-sing males in this species (Illes and Yunes-Jimenez, 2009; Hall et al., 2010). In the following, we discuss the relation of song development and song system development of males and females in order to validate the adult sexual dimorphisms.

Most surprisingly, during development, (1) the sex difference in the song system anatomy precedes the onset of song production and (2) song development progresses in a monomorphic way while the sexual neuroanatomical dimorphism is evident throughout the ontogeny (HVC and RA are 30–50% smaller in females than in males; **Figures 5A,C**) of the song system. The sex-specific vocal features (syllable repertoire, song duration) emerged only in adulthood. Thus, the main explanatory challenge of the present data concerns that female cordon-bleus develop their songs (that are comparable with those of males) with much less (ca. 30–50% less HVC and ca. 35–55% less RA) neurons than their male conspecifics. Either HVC and RA neuron numbers are the limiting factor for song development only in cases (females of certain species) in which song neuron numbers are below a certain threshold such as in the female zebra finch (Konishi and Akutagawa, 1985) and female Bengalese finches (*Lonchura striata* var. *domestica*) (Tobari et al., 2005; Gahr, unpublished observation), or female cordon-bleus differentiate song control circuits that differ qualitatively from those of their male conspecifics. In the first case, HVC and RA neuron numbers would be over-produced in case of the males, which leads to the question of evolutionary stability of this trait in light of developmental costs (Gil and Gahr, 2002). However, the regression of the size of HVC and RA neuron pools in the females of species in which females do not sing makes this conclusion unlikely.

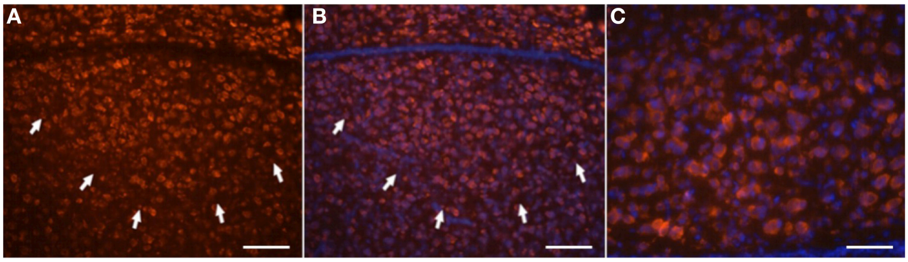

FIGURE 6 | Neuron-specific (Hu$^+$) labeling of HVC neurons. Hu-antibody labeling is red **(A)**, cell nucleus (Dapi) labeling is blue **(B,C)**. Double-labeled cells are identified as neurons **(B,C)**. **(C)** Shows a higher magnification view of **(B)**. Arrows indicate the ventral borders of HVC. Scale bar is 100 μm in A and B, and 40 μm in C.

TABLE 2 | Comparisons of HVC (A) and RA (B) neuron numbers of females and males revealed by Tukey *post-hoc* tests.

Age	20 dph	100 dph	Adult
(A) HVC NEURON NUMBERS			
20 dph	**0.0008**	**< 0.001**	**0.0189**
100 dph	**< 0.0001**	**0.0005**	**<0.0001**
Adult	**< 0.0001**	**0.01**	**<0.0001**
(B) RA NEURON NUMBERS			
20 dph	**0.0023**	**0.0221**	0.7640
100 dph	**<0.0001**	**0.0005**	**0.0159**
Adult	**0.0132**	**0.0147**	**<0.001**

P-values of female-female comparisons are in light-gray, those of male-male comparisons in dark-gray, and those of male-female comparisons are in the diagonal. Significant P-values are in bold. dph, age in days post-hatching.

Further (see below), both male and female cordon-bleus regulate the size of the HVC neuron pool during development (**Figures 5B,D**). Likewise, in species in which adult males and females sing identical songs (Gahr et al., 2008) or very similar songs (Gahr et al., 1998; Schwabl et al., 2015) or in which females and subordinate males sing the same song (Voigt and Gahr, 2011), there is nevertheless a strong sex difference of the song system anatomy. As the most parsimonious explanation we suggest that female cordon-bleus evolved HVC and RA circuits that function differently from those of the males to compensate for smaller song neuron pools in order to allow sensorimotor learning and the production of learned songs. Additional male-specific functions of HVC and RA next to singing are currently unknown. For example, the control of call exchanges by forebrain song areas (Ter Maat et al., 2014) is not male-specific (Trost et al., unpublished data), and therefore cannot account for the observed differences in HVC and RA anatomy.

Developmental Mechanisms of HVC and RA Size

Early sex difference in the number of song neurons preceding the onset of song production might be a general feature of estrildid finches (family Estrildidae) but not typical for other songbirds. However, since it occurs as well in juvenile canaries (Gahr et al., 1997), a species of the carduelid genus (family Fringillidae), early ontogenetic sex difference of the song system is likely an ancestral general songbird feature, due to the evolutionary history of the songbird suborder, as suggested previously (Gahr et al., 2008; Schwabl et al., 2015). In relation, sexually dimorphic song control areas were found in all species studied in detail (significant number of individuals) of various families of both the Passerida lineage (e.g., Estrildidae: zebra finch, Nottebohm and Arnold, 1976; Troglodytidae: bay wren, *Thryothorus nigricapillus*, (Brenowitz and Arnold, 1986); Ploceidae: forest weaver, *Ploceus bicolor*, Gahr et al., 2008); and the Corvida lineage (e.g., Corvidae: large-billed crows, *Corvus macrorhynchos*, Wang et al., 2009; Malaconotidae: slate-colored boubou, *Laniarius funebris*, Gahr et al., 1998; Icteridae: streak-backed oriole, *Icterus pustulatus*, Hall et al., 2010) of oscines as well as in the red-backed fairy wren (*Malurus melanocephalus*, Schwabl et al., 2015) of the basal oscine family Maluridae, according to the oscine phylogeny of Barker et al. (2004). However, the study of sex differences of the developing song system of further species with singing females, in particular of the basal australo-asian oscine lineages would be desirable.

Initial sex differences in the size of HVC and RA neuron pools of zebra finches are likely genetically determined (Wade and Arnold, 1996; Gahr et al., 1998; Agate et al., 2003) suggesting a similar explanation for the early sex differences of cordon-bleu song areas. The subsequent ontogenetic development of the song system might be linked to gonadal hormone activity although clear proof for this is missing in any songbird species except for the observation that androgen receptors are expressed in the developing HVC and RA (Gahr, 1996; Gahr and Metzdorf, 1999; Jacobs et al., 1999; Kim et al., 2004) and that estrogen receptors occur in the juvenile HVC (Gahr and Konishi, 1988; Gahr, 1996). Gonadal hormones are likely to promote the survival of song neurons to a developmental stage that allows the intra-song system connectivity to form, which is thought necessary for song learning and development. Further, song system development of zebra finches, starlings, and canaries is sensitive to pharmacological levels of testosterone and its estrogenic metabolite 17β-estradiol (Gurney and Konishi, 1980; Gurney, 1981; Weichel et al., 1989; Casto and Ball, 1996). Since the overall development of HVC and RA of male and female blue-capped cordon-bleu females was very similar (**Figures 5A,C**), similar mechanisms are likely to stimulate the increase and decrease in size of these brain areas during ontogenetic stages of males and females. It is, however, unlikely that the developing ovary would secret elevated testosterone levels, which in turn could facilitate song system differentiation of the females. For e.g., at 100 dph, HVC of female cordon bleus reached a size similar to that of adult males. Thus, the mechanisms controlling the developing song system of females (and males) need further investigations.

On the cellular level, changes of HVC and RA volume might be due to a combination of different forms of plasticity: neurogenesis, gliogenesis, dendritic sprouting, myelination, vascularization, synapse forming, pruning (synaptic, axonal), cell death (neurons, glia, endothelial cells), and regression (dendrites, vessels, glia) (for review: Stiles and Jernigan, 2010; Anderson, 2011). Of these, we estimated neuron numbers as a factor underlying developmental volumetric changes of HVC and RA of male and female cordon-bleus. The increase of HVC volume from 20 to 100 dph involved a strong increase in neuron numbers. In relation, protracted neurogenesis is a general event of the postnatal birdbrain (males and females) and recruitment of considerable numbers of new HVC neurons has been demonstrated during development of the HVC of male zebra finch (Kirn and DeVoogd, 1989; Kirn, 2010). Although only shown for males, it is conceivable that recruitment of newly born neurons occurs in both male and female HVC during post-hatching life, next to spreading out of early born HVC neurons (Gahr and Metzdorf, 1999). After 100 dph, the decline of HVC size of the females correlates with the reduction of HVC neuron numbers (**Figures 5A,B**), however, in males, HVC neuron numbers decline without changes of HVC size (**Figures 5C,D**).

In case of RA, there is a correlation between neuron number development and volume development in males and females. In zebra finches, most RA neurons are born before/around hatching and post-hatching neurogenesis seems to exclude the arcopallium (in which RA forms) of songbirds (Konishi and Akutagawa, 1990; Vellema et al., 2010). Since neuron numbers of cordon-bleus' RA increased after 20 dph, a large number of these neurons must be born much later as compared to the zebra finch, which might be plausible in light of the much longer ontogeny of the cordon-bleus (this study) as compared to the zebra finch (Immelmann, 1969; Konishi and Akutagawa, 1988). Alternatively, early born neurons might differentiate into RA neurons at a late time point. Thus, we need to consider that delineation problems of the brain areas (Gahr, 1990, 1997), i.e., the recognition of the outlines of RA (as well as of HVC for this argument) might have considerable effects on the estimated neuron numbers particularly in very young males and females.

However, future studies are needed to monitor various forms of plasticity, in particular neurogenesis and apoptosis, dendritic arborization, and gliogenesis, to confirm and extend the above considerations explaining the developmental change of HVC and RA volume of female and male blue-capped cordon-bleus. A future detailed study of the hormonal profiles of developing male and female cordon-bleus might show if gonadal hormones are important for sex-specific development of the song system in this species.

What are the Behavioral Consequences of the Increase and Decrease of HVC and RA Volume, Respectively Neuron Numbers of Male and Female Blue-capped Cordon-bleus?

Since we suggested above that the HVC and RA circuits of males and females function in a sex-specific way, we do not consider sexual dimorphisms in volume and neuron numbers for this discussion, i.e., we focus on the relationship of developmental changes of brain and song behavior. In either sex, the increase of volume of HVC and RA between 20 and 100 dph (and of HVC neuron numbers) might correlate with a reduced variability in singing that is reflected in recurring syllables, i.e., a measurable syllable repertoire at 100 dph (**Figures 1**, **2A,B**, **5**). Further, around this age the animals might produce the largest number of different syllable sequences although this could not be quantified due to insufficient syllable stereotypy earlier on. Thus, the increase of HVC and RA size till 100 dph might correlate with the production of an increased number of motor patterns. Although these syllables have a level of stereotypy that allows their classification, this stereotypy is certainly lower compared to songs uttered by older females and males (**Figures 1**, **2C**). Between 100 dph and adulthood syllable numbers decrease in both sexes, the stereotypy of syllable harmonicity increases in both sexes, and song duration decreases in females. We could view these song developments as increased stereotypy and decreased number of motor pattern that emerges in relation with decreasing HVC volumes (females) and RA volumes (males and females) of cordon-bleus. Further, increased stereotypy and decreased number of motor pattern would correlate with decreasing numbers of neurons of HVC and RA after 100. A decreasing rate of adult neurogenesis and newly recruited HVC neurons was thought to correlate with increasing stereotypy of the song of male zebra finches (Pytte et al., 2007).

A relationship of the anatomical development of forebrain song control areas and of song learning has been hypothesized before based on male zebra finches and male canaries (for review: Kirn, 2010). However, as detailed above, in the cordon-bleus a causal relationship between anatomical changes of HVC and RA and song development would be area- and sex-specific. Alternatively, such complicated relationships might indicate that—like in human brain and cognitive development (Stiles and Jernigan, 2010)—the maturation and deterioration of specific brain regions is an insufficient explanatory mechanism for song development of birds. Further, late developmental changes of the syllable repertoire in female cordon-bleus show that females do not just lose syllables but seem to acquire new syllables and replace old ones at late puberty/early adulthood (**Figure 1**). Likewise, male cordon-bleus change their repertoire very late (after 250 dph) at an age when the size of vocal areas already reached adult size. Thus, sexual selection or the need for social song adjustment rather than brain autonomous maturation processes might play a role for the decrease in repertoire size and song duration in female cordon-bleus and for the change of repertoire composition in both males and females, independent of gross morphological changes. On more ultimate terms, developmental overproduction of song patterns followed by repertoire attrition has been described for several species and has been interpreted in the context of social adjustments of repertoires (e.g., Marler and Peters, 1982; Nelson, 1992; Geberzahn et al., 2002). Future long-term monitoring of cordon-bleus shall show if females and males of this species classify as open-ended vocal learners in contrast to other Estrildid finches such as the zebra finch.

CONCLUSION

In summary, the comparative study of song system development and song development of the blue-capped cordon-bleus, a species in which males and females sing learned songs sheds new light on the potential relationships between these processes. In particular, sex-specific song system development seems not strictly related to sex-specific song development. This suggests that medium to small sex differences in the phenotype of the song system are not predictive concerning the degree of song monomorphy of songbirds. The study of further species in which males and females sing very similar songs (Odom et al., 2014) should show if the dissociation of neuroanatomical and song development is a general feature of songbirds.

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FEMALE SONG AND OTHER DISPLAY TRAITS

Female superb fairy-wrens have complex songs and blue tails, but the two ornamental traits are not correlated.
Photo: Ashton Claridge and Sonia Kleindorfer

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Female Song Occurs in Songbirds with More Elaborate Female Coloration and Reduced Sexual Dichromatism

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Elaborate plumages and songs in male birds provide classic evidence for Darwinian sexual selection. However, trait elaboration in birds is not gender-restricted: female song has recently been revealed as a taxonomically-widespread trait within the songbirds (oscine Passerines), prompting increased research into likely functions and social/ecological correlates. Here we use phylogenetically-informed comparative analysis to test for an evolutionary association between female song and plumage color elaboration in songbirds. If there is an evolutionary trade-off between signaling modes, we predict a negative correlation between acoustic and visual elaboration. This trade-off hypothesis has been commonly proposed in males but has mixed empirical support. Alternatively, if song and plumage have similar or overlapping functions and evolve under similar selection pressures, we predict a positive correlation between female song and female plumage elaboration. We use published data on female song for 1023 species of songbirds and a novel approach that allows for the reliable and objective comparison of color elaboration between species and genders. Our results reveal a significant positive correlation between female colorfulness and female song presence. In species where females sing, females (but not males) are on average more colorful—with concomitantly reduced average sexual dichromatism. These results suggest that female plumage and female song likely evolved together under similar selection pressures and that their respective functions are reinforcing. We discuss the potential roles of sexual vs. social selection in driving this relationship, and the implications for future research on female signals.

Keywords: female song, multimodal signaling, oscine, Passeriformes, plumage coloration, trade-off, trait correlation

INTRODUCTION

Elaborate male traits often provide evidence for classic sexual selection (Darwin, 1871), but the possession of elaborate traits by females is less well understood. A traditional perspective holds that female trait elaboration is non-adaptive, perhaps detrimental, and results from "shared genetic architecture" with males (Darwin, 1871; Lande, 1980; see Tobias et al., 2012 for review). While current evidence does suggest that female ornamentation is correlated with conspecific male ornamentation to some extent (Bonduriansky and Chenoweth, 2009; Dale et al., 2015a) the view that elaborate female traits are purely non-adaptive pleiotropic effects has been strongly refuted by recent research.

Bird song and elaborate plumage are often considered costly traits (Song: Oberweger and Goller, 2001; Nowicki et al., 2002; Berg et al., 2005; Schmidt et al., 2013; but see Gil et al., 2006. Plumage: McGraw et al., 2002; Walther and Clayton, 2005; Simpson et al., 2015). In females, both traits can be evolutionarily labile with respect to the conspecific male phenotype. First, female plumage changes have played a greater role than male changes in the evolution of dichromatism in New World blackbirds (Icteridae; Irwin, 1994; Hofmann et al., 2008; Price and Eaton, 2014), tanagers (Thraupidae, Burns, 1998) and fairy-wrens (Maluridae, Johnson et al., 2013) and this pattern was shown to hold true for the order Passeriformes as a whole (Dale et al., 2015a).

Second, female song has been recovered as the ancestral condition of songbirds (Odom et al., 2014), indicating that multiple losses of female song have occurred over evolutionary time despite conspecific males retaining the trait (Price, 2015). This shows that female traits are not necessarily tightly constrained by the male phenotype, but are able to evolve rapidly and independently.

Furthermore, female-specific functions of song and plumage ornamentation have been resolved for a growing number of species, revealing these traits to be potentially adaptive for females. For instance, female song has been shown to function in territory and resource defense, mate attraction, mate defense, and pair-bonding (Searcy and Yasukawa, 1995; Langmore et al., 1996; Rogers et al., 2007; Brunton et al., 2008; Templeton et al., 2011; Hall et al., 2015). Female coloration is attractive to mates in some species (Amundsen et al., 1997; Smiseth and Amundsen, 2000; Murphy et al., 2009a,b) and frequently has roles in female-female competition for non-sexual breeding resources (Tobias et al., 2012; Morales et al., 2014).

If elaborated female traits are adaptive, this raises the question: what is the evolutionary relationship between female song and plumage color elaboration? We outline three hypotheses regarding the evolution of multiple elaborate traits: the traits may evolve "in a concerted fashion, in an antagonistic fashion, or in ways unrelated to each other" (Shutler and Weatherhead, 1990). In our context, these options are detailed as follows:

(i) The "trade-off" hypothesis (Darwin, 1871) argues that if two modes of signaling are both costly to produce or maintain, then selection might favor doing one thing well rather than two things badly. This predicts an evolutionary trade-off (an inverse correlation) between signaling modes (Shutler and Weatherhead, 1990). Thus, species with female song are predicted to have less colorful females on average than species lacking female song.

(ii) The "reinforcing signals" hypothesis (inspired by the "redundant signal" hypothesis of Møller and Pomiankowski, 1993) proposes that the two ornamental traits have overlapping, reinforcing functions, acting in concert to convey the condition or status of the signaler. Whereas either trait on its own provides a partial indication of signaler condition or status, in combination the multiple ornaments enable a more accurate assessment by rivals or mates. This predicts a co-evolution of the two traits; that is, a positive correlation between song and plumage color elaboration in females. Thus, species with female song are predicted to have more colorful females on average than species lacking female song.

(iii) The "multiple messages" hypothesis (adapted from Møller and Pomiankowski, 1993) posits that the two traits reveal different information about their bearers. This implies that the characters are driven by different selective pressures, and therefore elaborate vocal and visual sexual signals should evolve independently. Thus, there should be no difference in female color elaboration between species with female song, and those without female song.

Current evidence is inconclusive regarding the three hypotheses. In line with the trade-off hypothesis, an inverse relationship has been found between male plumage brightness and song complexity in cardueline finches (Badyaev et al., 2002). But in support of the reinforcing signals hypothesis, a positive correlation has been found between song length and number of colored patches among Asian barbets (Gonzalez-Voyer et al., 2013), and a positive correlation between the degree of dichromatism and time spent singing among wood warblers (Shutler and Weatherhead, 1990). Finally, Ornelas et al. (2009) found no relationship between dichromatism and song complexity among trogons, and Mason et al. (2014) found no correlation between song and plumage complexity among the tanagers. These conflicting results likely reflect biological and evolutionary differences among focal taxa, as well as methodological differences in how song and plumage were quantified (Mason et al., 2014). Furthermore, all these studies focus on males. The possibility of a generalized macroevolutionary association between multiple ornamental traits in females has not yet been investigated.

In this study we test for an evolutionary correlation between female song and plumage elaboration (male, female and dichromatism) across the songbirds (i.e., Oscines; order Passeriformes, suborder Passeri). We perform phylogenetically-informed comparative analysis using song and plumage data from repositories supplemented with additional data gleaned from the literature.

METHODS

Scoring Female Song

We compiled data describing the presence or absence of song in male and female songbirds (Oscines) from Odom et al. (2014) and del Hoyo et al. (2004–2011). We gave each species one of four scores according to the criteria of Odom et al. (2014, full details therein). Scores included: "present," both males and females of the species sing; "absent," only the male sings; "songless," neither sex sings; or "not enough information" if we could not reliably make a designation. (Note that no species where only females sing has been described.) Out of all 4814 songbird species, 1314 had sufficient information on song to reliably score the species. Because the lack of female song in songless species might be the product of different selection pressures than in species with male song, we omitted songless species (291 species) from our analysis. Our final species pool included 1023 singing species comprised of 656 species where both sexes sing (64%), and 367 species where only males sing (36%).

Scoring Plumage Elaboration

Plumage color scores for the 1023 songbird species were obtained from Dale et al. (2015b). Briefly, for both sexes of each species of passerine (Order: Passeriformes), the mean red, green and blue (RGB) values on 3 dorsal and 3 ventral patches were measured using digital image processing software (Valcu and Dale, 2013) on scanned images from handbook plates. For each patch of each sex of each species, it was determined how "male-like" that patch is by scoring the proportion of males in the nearest 1% of similarly colored patches in other species. The method results in scores where low values correspond to males or females with drab, classically-"female-like" plumage, and high scores correspond to males or females with elaborate, classically-"male-like" plumage. This approach is transferable to other color quantification methods. There is a high correlation between scores determined with handbook plates vs. analogous scores determined with UV-VIS (ultraviolet to visible) reflectance spectra from museum specimens (Dale et al., 2015a). This result provides critical validation of the method because although human and avian vision have considerable overlap (Badyaev and Hill, 2003; Seddon et al., 2010), birds can also see UV light not visible to humans (Cuthill, 2006). See Dale et al. (2015a) for detailed methodology. Sexual dichromatism was calculated for each species as the male plumage color score minus the female plumage score.

Trait Correlation Test

To test for an evolutionary correlation between female song and female plumage elaboration, we first performed Pagel's correlation test (Pagel, 1994), in R 3.1.2 (R Development Core Team, 2014) using the "geiger" and "phytools" packages (Harmon et al., 2008; Revell, 2012). The Pagel test controls for phylogenetic relatedness and requires no designation of independent and response variables. We assigned a song character state and a plumage character state to each tip of a phylogeny, and tested the null hypothesis that the two traits had evolved independently. As the Pagel test requires both traits to be binary, plumage scores were binned into binary characters according to an arbitrary cut-off, which was moved in integer increments from 35 (1022 of 1023 species with female plumage elaboration present) to 71 (1 of 1023 species), to study the sensitivity of the correlation test to changes in plumage cutoff value. The phylogenies used for this analysis were obtained from the Hackett backbone (Hackett et al., 2008) supertrees at http://birdtree.org (Jetz et al., 2012).

MCMCglmm Analysis

To estimate the strength of the correlation between female song presence and female plumage elaboration, we fit a multivariate generalized linear mixed model using the "MCMCglmm" package (Monte Carlo Markov Chain generalized linear mixed model; Hadfield, 2010) in R (version 3.1.2). MCMCglmm allowed us to fit a model which had a response vector that contained a mixture of Gaussian and non-Gaussian distributed variables. Female plumage elaboration is a continuous measure (see above); hence we assumed a Gaussian error distribution. For female song, the response vector contained binary presence scores ($0 = $ female song absent, and $1 = $ female song present) and accordingly we assumed a Bernouli error distribution and used a logit link function. Fixed effects in our model included female plumage elaboration, the presence of female song, and male plumage elaboration as a covariate. Phylogeny was fit as a random effect using the methods described in Hadfield and Nakagawa (2010) to calculate the inverse numerator relationship for phylogenetic effects. For the phylogenetic effects we also allowed separate random intercepts for female song and female plumage elaboration and a non-zero covariance between these two traits by assuming an unstructured variance-covariance structure.

Priors for the location effects were diffuse about zero and had a large variance (10^8). For the variance components we used priors conforming to a scaled non-central F-distribution (Gelman, 2006) with the location parameter equal to zero. The scale parameter for female color elaboration was equal to half of the phenotypic variation in female color elaboration, and for female song the scale parameter was equal to $p(1 - p)$, where p is the mean probability of female song across the dataset. For the residual covariance matrix we assumed an inverse-Wishart distributed prior for female coloration. For female song (which is a binary trait), it is not possible to estimate a residual variance, so we fixed the prior at a value of 1 (Hadfield, 2014). The MCMC chain had 20,600,000 iterations, with a burn-in of 600,000 and a thinning interval of 20,000, resulting in $\sim$1000 samples of the posterior distribution of the parameters. Model fit was confirmed by ensuring that autocorrelation was low and the trait means lay within the 95% highest posterior density (HPD) intervals of the posterior predictive distribution of each trait. To incorporate some of the uncertainty in the phylogenetic relationships among bird species, we applied the statistical model described above to 10 different phylogenetic trees randomly selected from http://birdtree.org (Jetz et al., 2012). Finally, we examined the convergence of the phylogenetic variances and covariances estimated from the 10 models (each using different trees, and therefore with different numerator relationship matrices for the phylogenetic effects), with the Gelman and Rubin (1992) diagnostic, R. For these 10 trees, the point estimate was $R = 1.2$ indicating moderate convergence. This phylogenetic uncertainly is incorporated in all the estimates of the posterior means and the HPD intervals we present.

To assess the significance of the phylogenetic correlation between female song (FS) and female plumage elaboration (FP), we first calculated the posterior distribution of the correlation using $cor_{(FS,\ FP)} = cov_{(FS,\ FP)} \div (var_{FS} \times var_{FP})^{0.5}$

If the 95% HPD intervals of the posterior distribution of the correlation did not overlap zero, we interpreted this as evidence for a significant phylogenetic correlation between female plumage elaboration and female song. Similarly, to assess the significance of the association between male color elaboration and our two female traits, we tested whether the 95% HPD intervals of the fixed interactions between male plumage and female plumage elaboration, as well as between male plumage and female song, overlapped zero.

RESULTS

Female Song in Relation to Plumage Color Elaboration

For many species in our sample, males and females have similar plumage elaboration scores (**Figure 1A**, points lying along the diagonal). However, there are also many sexually dichromatic species where the male is more colorful and the female is more drab (**Figure 1A**, points in the upper left of the distribution). Overlaying the density of female song presence on this plumage elaboration scatter (**Figure 1B**) reveals that female song presence is most concentrated amongst species with high female plumage scores and reduced average sexual dichromatism.

Phylogenetic Distribution

The phylogenetic distribution of the co-occurrence of female elaboration traits is visualized in **Figure 2**, where color-coded species tips correspond to the presence/absence of female song and female plumage elaboration. Instances where both traits co-occur (257 species, 25% of the total sample) are not concentrated within one or a few clades but are dispersed throughout the tree, suggesting many separate origins of the co-evolution of these traits (**Figure 2**). Wide phylogenetic dispersion persists when higher plumage elaboration cutoff values or different trees from Jetz et al. (2012) are used. Also numerous and widely dispersed around the tree are species with only one trait or the other: female song but not female plumage elaboration (399 species, 39% of the total sample); female plumage elaboration but not female song (72 species, 7% of the total sample), and neither trait present (295 species, 29% of the total sample).

Pagel Correlation Test

The Pagel test provides strong support for correlated evolution of female song presence and female plumage elaboration for all plumage cut-off values between 41 and 54 (**Figure 3**, $P < 0.0001$). This is a large range by comparison to the entire range of female plumage scores (35–71), and even at a cut-off as high as 60, the correlation is still statistically significant at the 5% level. The presence of an evolutionary association between female song and female color elaboration is therefore highly robust to alternative cut-off values used to categorize female coloration as elaborated.

MCMCglmm

Despite the phylogenetic uncertainty introduced by using 10 randomly selected phylogenetic trees from Jetz et al. (2012), we found a strongly significant evolutionary correlation between female plumage elaboration and the presence of female song (**Figure 4**, mean $cor_{(FS,\ FP)} = 0.402$, 95% HPD = 0.220–0.583). Accounting for evolutionary relationships among bird species we found that male and female color elaboration were positively associated (posterior mean = 0.472, 95% HPD = 0.422–0.527), confirming the apparent pattern seen in **Figure 1**. In addition there was a positive, but not statistically significant, relationship between the degree of male color elaboration and the presence of female song (posterior mean = 0.044, 95% HPD = $^{-}0.014$–0.102).

DISCUSSION

We used phylogenetically-informed analysis to reveal the relationship between female song presence and plumage elaboration across the songbirds. Our study shows that (1) female song is more concentrated amongst species with elaborated (classically “male-like”) female plumage, (2) the co-occurrence of female song and female plumage elaboration is widely dispersed across the songbird phylogeny, suggesting many independent origins of this evolutionary association, (3) Pagel correlation tests demonstrate clear support for an evolutionary correlation between female song and female plumage color elaboration, and

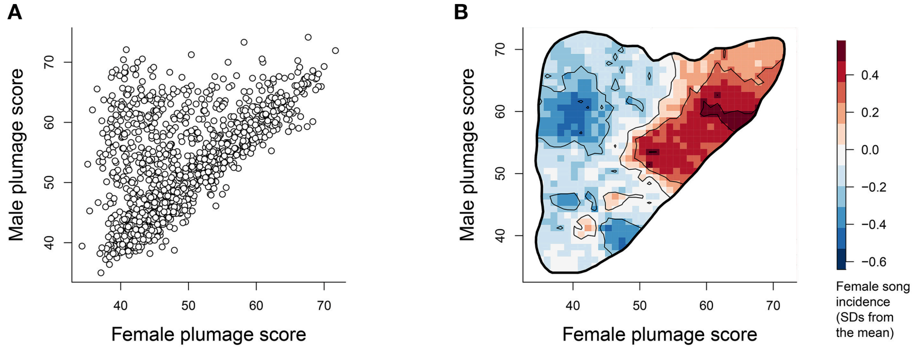

FIGURE 1 | (A) Male plumage score vs. female plumage score for each species ($N = 1023$). **(B)** Contour map depicting the average of the (scaled) female song values overlaid within the 99.8% volume contour of the male vs. female plumage score distribution. Values within the plot were calculated by superimposing a 50 × 50 grid over the scatter occurring between 30 and 80 and then calculating at each grid point the mean female song scores of the closest 3% of species. The units represent standard deviations away from the mean incidence of song, with redder values indicating increasingly higher occurrence of female song.

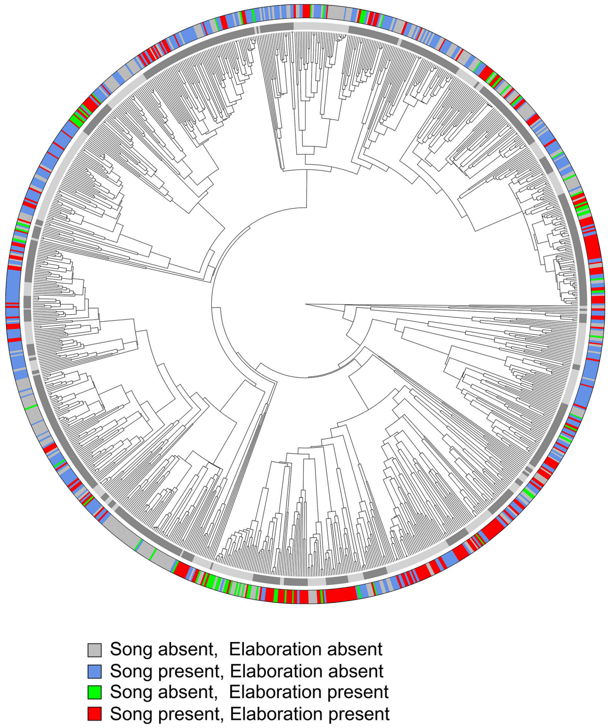

FIGURE 2 | Phylogenetic distribution of female song and female plumage elaboration. Plumage elaboration is here treated as a binary character with cutoff = plumage score of 50. Red labels represent species where female song and female plumage elaboration (plumage score >50) co-occur. The inner ring of alternating gray tones depicts boundaries of the 86 oscine families in our sample.

(4) estimates from MCMCglmm models suggest that the presence of female song accounts for 16.2% ($R = 0.402$) of the variation in female plumage elaboration (and vice versa).

Mason et al. (2014) provided the largest comparative study of multimodal sexual signaling to date, finding no relationship between plumage elaboration and song elaboration across the males of 301 tanager species (Thraupidae). In contrast, our results show a clear positive relationship between female song and female plumage color elaboration across the songbirds. The difference between our results and those of Mason et al. could stem from a number of factors. First, Mason et al. consider only male elaboration. The selective pressures acting on males and females are not necessarily equivalent, and so female traits may exhibit a different relationship to each other than do male traits (Tobias et al., 2012). Second, we use different methods of scoring song and plumage elaboration; notably, the song data of Mason et al. are quantitative, whereas our data are presence/absence. Finally, there are differences in scale between our studies. The presence of negative or non-significant relationships within some families such as the Thraupidae does not preclude a positive correlation at the broader taxonomic level of the songbirds as a whole. These considerations apply equally to other studies of multimodal signaling which find a negative relationship or no relationship between song and plumage (Badyaev et al., 2002; Ornelas et al., 2009).

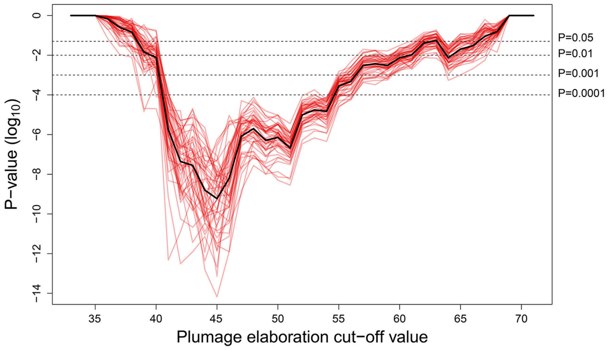

FIGURE 3 | Pagel correlation test *P*-values (Pagel, 1994) at all possible plumage elaboration cutoff values, for each of 50 phylogenetic trees from http://birdtree.org (Jetz et al., 2012). The black line indicates the mean of the 50 runs (red lines). Evidence of correlated evolution is strong (<0.0001) for all plumage cut-off values between 41 and 54.

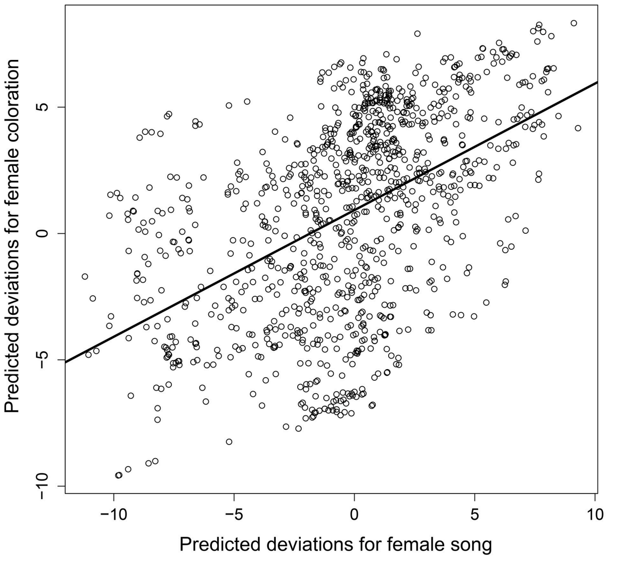

FIGURE 4 | Plot of best linear unbiased predictions of the phylogenetic association between female song and female plumage color elaboration. Points indicate the predicted phylogenetic deviations of each species (i.e., the 1023 species tips within the pruned phylogenetic tree) for the two female display traits, and solid line denotes the posterior mean phylogenetic correlation between female song with female plumage.

Our results are most consistent with the "reinforcing signals" hypothesis; a positive correlation between female song and female plumage elaboration is expected if the two signaling modes tend to have reinforcing, overlapping functions, and thus have co-evolved together in response to similar selective pressures. Females may use song and plumage as a multimodal signal to reinforce the reliability and/or potency of the message to receivers.

A central tenet of sexual selection theory is that males and females often differ in their routes to reproductive success; for males, competition for mates is paramount, whereas for females, access to resources that affect fecundity is thought to be of greater importance (Rosvall, 2011). Therefore, the primary selection operating on females is likely to be non-sexual "social selection" for ecological or social resources, such as foraging territories, nest sites and paternal investment (West-Eberhard, 1979, 1983; Tobias et al., 2012). Such a view would suggest that a key function of both female song and plumage color elaboration may be signaling the female status (or resource-holding potential: Searcy and Nowicki, 2005) to competitive rivals, a prediction that is borne out in a number of studies (Tobias et al., 2012). This is not to deny that sexual selection for elaborate female traits may also be important (Clutton-Brock, 2007; Rosvall, 2011; Clutton-Brock and Huchard, 2013); indeed, the same ornaments can perform both sexual and (non-sexual) social functions (Kraaijeveld et al., 2004).

The co-occurrence of both visual and acoustic indicators of status (or resource-holding potential) likely reinforces the overall message and facilitates effective communication under different signaling scenarios. Song can be communicated over longer distances, without a clear line of sight; and as sound is propagated radially (Fahy and Gardonio, 2007), vocal communication does not depend on precise directionality of the signaler in relation to the receiver. Song is amenable to rapid temporal changes, conveying the short-term intentions of the individual and encoding complex information about signaler identity. Plumage coloration, by contrast, is a more permanent feature (though birds may be able to mediate plumage display behaviorally). In general, plumage coloration is a more direction-sensitive close-range signal than song. Thus, to signal quality and ward off rivals, selection could favor song for long-range broadcasting and plumage elaboration for close encounters, even if the message and intended receiver are the same.

If differing signaling scenarios promote female song or plumage elaboration differentially, this might explain the many species in our sample with only one trait or the other. Given that 16.2% of variation in female plumage elaboration is explained by female song presence (and vice versa), a remaining 83.8% of variation in each trait is thus attributable to other factors. That is, the relationship between female song and female plumage elaboration is complex and likely mediated by additional effects of, for example, habitat type, predation risk, territory size, and social structure. Closed habitat might strongly favor female song for effective communication if the range and efficacy of visual signaling is impaired. Or, for species experiencing high predation, elaborate female plumage may impose too great a risk around the nest (Martin and Badyaev, 1996) and be selected against. Or, in colony-living species where individuals remain in close proximity, close-range visual signaling may be sufficient for female signaling needs, making female song redundant. In short, there is much work left to do in identifying and quantifying the factors that contribute to visual and acoustic ornamentation in female songbirds.

If song and elaborate plumage are both costly (e.g., Oberweger and Goller, 2001; McGraw et al., 2002), as expected if the signals are "honest" indicators of aspects of female quality, then why have we not observed a trade-off between signaling modes? There are a number of potential explanations. First, while our results demonstrate that *presence* of female song does not trade off with female plumage elaboration, we lack information on the degree of female song *elaboration* (e.g., in terms of repertoire size, vocal agility, and time spent singing) and thus we cannot evaluate whether there is some degree of trade-off of resource allocation between modes. As more studies focus on quantifying the vocal performance of female birds, addressing whether singing females with bright plumage have "cheaper" songs than those with drab plumage will be practicable. It is also conceivable that when comparing across species, trade-offs might not manifest if different species are selected to invest different levels of resources into the overall message.

Another possibility is that resources involved in song and plumage development are not limiting for species where status signaling is strongly favored by selection. Rather than functioning as condition-dependent indices or handicaps, the honesty of these signals may instead be socially enforced, with cheaters (i.e., individuals who signal having greater status than they actually have) being punished through increased aggression by conspecifics (Tibbetts and Dale, 2004; Tibbetts and Izzo, 2010). The social cost in such cases will depend on how accurately the signal reflects true quality of the individual, rather than the number of ornaments involved in the signal *per se*. That is, under social costs, female song and plumage elaboration may be no more costly to produce than either trait alone, in which case we would not expect a trade-off between traits.

CONCLUSION AND FUTURE DIRECTIONS

We have demonstrated strong evidence for a positive co-evolutionary relationship between plumage elaboration and song in female songbirds, a result which supports an overlapping function of the two traits (i.e., the reinforcing signals hypothesis). We have suggested, in light of current selection theory (Tobias et al., 2012), that the primary context for this multimodal signaling is non-sexual social competition for ecological or social resources, and that the different signaling ranges of plumage and song may have favored the evolutionary maintenance of both traits. Our finding raises several questions for future research, including: (1) Does this pattern hold for the sub-oscines? Though generally poorer singers than the songbirds, and lacking vocal learning (but see Kroodsma et al., 2013), many sub-oscines vocalize for mate attraction and territorial defense (Chelén et al., 2005) and thus may be subject to similar evolutionary pressures. (2) Are female ornaments gained and lost more frequently than male ornaments (Kraaijeveld, 2014) and is there a consistent order of female trait evolution? That is, do gains (or losses) of song follow gains (or losses) in plumage elaboration, or vice versa? And (3), what are the social and ecological drivers of the evolutionary association of female song and female plumage elaboration? It is our hope that the patterns reported in this study contribute to future research on the functions of both visual and acoustic ornamentation in females.

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AUTHOR CONTRIBUTIONS

Conceived of the study: WW, JD, and DB; collected the data: JD, MV, WW; analyzed the data: WW, JD, JA, DT; wrote the paper: WW, JD, and JA with input from the other authors.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: http://journal.frontiersin.org/article/10.3389/fevo.2016.00022

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Multiple Ornaments—Multiple Signaling Functions? The Importance of Song and UV Plumage Coloration in Female Superb Fairy-wrens (*Malurus cyaneus*)

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Showy ornaments are considered as outcomes of sexual selection processes. They provide a "badge of status" to impress conspecific rivals or potential mating partners. Single ornaments may signal attractiveness or individual quality, yet many species display multiple ornaments. There are several hypotheses that explain the existence of multiple ornaments, suggesting that different ornaments serve as different information sources. They may provide either additive or redundant information on the same quality traits, or are simply evolutionary leftovers with no further relevant information. Although, females of many species display elaborated traits, most studies regarding multiple ornaments focus on males. However, given that in many species females also display multiple ornaments, the question about their functional significance arises. To understand the existence of female multiple ornaments we investigated ornamental features of female Superb Fairy-wrens (*Malurus cyaneus*), focusing on song and variation in plumage characteristics. Female Superb Fairy-wrens produce complex solo songs, for territorial defense, and have bright blue tail feathers. We examined the relationships between song and plumage coloration characteristics in relation to female quality parameters to investigate whether, and to what extent existing hypotheses on multiple ornaments in males may also apply to females. Based on song recordings and spectrometric measurements of UV-coloration of tail feathers, we derived a series of different song and plumage parameters. Our results indicate interrelationships between the song length (total number of elements in female song) and female body size, but not UV-coloration. Interestingly, song complexity (number of different elements in female song) did not correlate with morphological parameters, UV-Chroma and song length, respectively. This suggests that (i) song and plumage characteristics evolved independently and (ii) even within one trait, namely song, multiple signaling should be considered. To our knowledge, this is the first study investigating multiple traits in female songbirds, raising the idea that multiple signaling of sexually selected traits is not restricted to males only.

Keywords: female, passerines, plumage coloration, song elaboration, multiple signals

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INTRODUCTION

Showy plumage characteristics and elaborated song in passerines are known to be typical male traits, shaped by sexual selection (Andersson, 1994). There is strong evidence that both traits signal individual quality and are therefore involved in mate choice, as well as competitive interactions (Burley, 1986; Searcy and Andersson, 1986; Andersson, 1994; Nowicki and Searcy, 2004; Hoi and Griggio, 2008). However, the expression of colorful plumage and elaborated song is not restricted to males; females can also display these traits (Webb et al., 2016). As previously stated by Langmore (1998) and Amundsen (2000) this phenomenon has largely been ignored until recently and these traits were either regarded as being the consequence of genetic correlation with male ornamentation, functionless, or a result of physiological abnormalities (Lande, 1980; Amundsen, 2000). However, a growing number of studies focused on the evolution of female ornamentation, including plumage and song characteristics (Amundsen et al., 1997; Langmore, 1997, 1998; Amundsen, 2000; Garamszegi et al., 2007; Doutrelant et al., 2008; Mahr et al., 2012; Tobias et al., 2012; Webb et al., 2016).

Several studies have revealed that female choice can be based on several different traits that signal male quality, such as morphological and behavioral traits (Burley, 1981; Johnstone, 1996; Lozano, 2009; Dolnik and Hoi, 2010; Hoi and Griggio, 2012). Multiple traits may (i) act as amplifiers by offering the same information, (ii) have an additive effect whereby the information of several traits complement each other or, (iii) provide different information e.g., about different qualities of the bearer (Burley, 1981; Grafen, 1990; Zuk et al., 1990, 1992; Johnstone, 1995, 1996; Marchetti, 1998; Rivera-Gutierrez et al., 2010). For example bird song and plumage are traits that can signal the same or different information and both traits are driven by sexual selection in males and females (Lande, 1980; Andersson, 1994; Amundsen et al., 1997; Langmore, 1998; Amundsen, 2000; Ball and MacDougall-Shackleton, 2001; Garamszegi et al., 2007; Hegyi et al., 2007, 2008; Cardoso and Hu, 2011). However, the interaction between both traits has hardly been investigated in females (Garamszegi et al., 2007; Webb et al., 2016).

Although, song and plumage traits may carry the same information, these traits can act on different time and spatial scales (Taff et al., 2012). Song usually acts as a long distance signal whereas plumage ornaments act as a short distance signal. When both signals carry the same information, one would predict a positive relationship in the expression of the traits. Alternatively, the expression of both ornamental features might underlie different mechanisms and require different preconditions to maintain an honest signaling function. Furthermore, different production costs may arise, which may consequently represent different qualities. In this case, one would predict that trade-offs between both traits could result in either negative correlations between traits or independent development of different traits, like song and plumage characteristics. However, to our knowledge this trade-off has only been investigated on a phylogenetic scale (Badyaev et al., 2002; Mason et al., 2014; Soma and Garamszegi, 2015).

Whether this also applies to females has, to our knowledge, only been investigated in two comparative studies, focusing on song and plumage development in passerine species. Garamszegi et al. (2007) suggested that singing behavior often occurs in the presence of carotenoid based ornamentation, which is supported by very recent findings from Webb et al. (2016). This positive association might indicate that both traits are generally used in a similar or the same functional context and hence carry the same information content. Nevertheless, only a few case studies examined the interaction between both traits within breeding populations in male songbirds (Møller et al., 1998; Chiver et al., 2008; Taff et al., 2012), and to our knowledge, no study on female birds exists so far. In this study we used the female Superb Fairy-wren (*Malurus cyaneus*) to examine (i) the signaling function of song characteristics and plumage coloration and (ii) the interaction between these two female ornamental features. To determine whether these ornaments reflect female quality we used female body size and body condition as covariates.

The Superb Fairy-wren is an ideal model species to answer our questions because both males and females sing solo chatter songs year-round for territorial defense (Cooney and Cockburn, 1995; Cain and Langmore, 2015), and the structure and complexity of female chatter song is similar to male chatter song (Kleindorfer et al., 2013). Mate attraction may be a secondary function of male chatter song (Dalziell and Cockburn, 2008), but to our knowledge there is no study investigating whether this function applies to female song. In contrast to song, Superb Fairy-wrens have a strong sexual plumage dichromatism. Whereas males have bright blue plumage, females are more cryptic, displaying only an orange lore and a blue tail that reflects in the UV range (own data represented in the Supplementary Material). Maluridae are sensitive to UV and females frequently wave their tail during foraging and social interactions (own unpublished data). This raises the question whether the UV reflecting tail of females is a sexually selected trait (Ödeen et al., 2012).

Previous studies demonstrated a decrease of UV reflectance in worn feathers and from dust accumulation (Örnborg et al., 2002; Zampiga et al., 2004; Griggio et al., 2010, 2011). There is a trade-off between the removal of ectoparasites and dirt from feathers, preening and activities like foraging or increased vigilance against predators (Redpath, 1988; Cucco and Malacarne, 1997; Shawkey et al., 2003; Kapun et al., 2011; Moreno-Rueda and Hoi, 2012). Interestingly there is evidence that similar mechanisms also apply to song features, in particular song rate, which is regarded as a highly variable trait depending on current physiological condition and time of the reproductive cycle (Gil and Gahr, 2002). Therefore, both plumage maintenance and singing behavior force individuals into a trade-off that individuals in poorer condition cannot afford, being forced to invest either more in one or equally, but less in both traits (Andersson et al., 2002).

In many songbird species, song complexity is regarded as stable over the year. The ability to produce complex songs can be an honest signal of quality, because during the development of the neural song system, the expression of neuronal structures and development of the syringeal muscles can be affected by early developmental stress such as under-nourishment (Nowicki et al., 2000; Spencer et al., 2003; Buchanan et al., 2004; Nowicki and Searcy, 2004). Hence, in contrast to plumage characteristics, song complexity can be regarded as less sensitive toward the change of individual condition after the crystallization and determination of singing behavior (Gil and Gahr, 2002).

Our study focuses on the relationship between song complexity and the number of elements females are using (rather than song rate) and UV-reflectance in the blue tail-feathers of female Superb Fairy-wrens. Given both traits may provide different information the question arises whether females use multiple traits to signal quality and condition to male and female conspecifics. Studies investigating relationships between multiple traits within populations are rare and to our knowledge this is the first study focusing on the relationship between features of song, plumage coloration, and morphological traits in a female passerine.

METHODS

Study Sites

The study was carried out during the breeding season between September and November 2012 and 2013 at three study sites on Kangaroo Island: Flinders Chase National Park (35°54′S, 136°47′E), Vivonne Bay Conservation Park (36°00′S, 137°09′E), and Kelly Hill Conservation Park (35°97′S, 136°90′E) and at two study sites on the mainland in South Australia (SA): Cleland Wildlife Conservation Park (35°05′S, 138°41′E) and Newlandhead Conservation Park (35°37′S, 138°29′E). All study sites and territories were chosen on the basis of long term monitoring of Superb Fairy-wren populations, conducted by the BirdLab at Flinders University (Colombelli-Négrel et al., 2010; Kleindorfer et al., 2013).

General Methods

All birds were caught with mist-nets using conspecific playback stimuli and banded with numbered aluminum rings provided by Australian Bird and Bat Banding Scheme (ABBBS) and a unique combination of darvic color rings. Standard measurements of the flattened wing chord length and tail length (to the nearest 0.5 mm) were taken with a ruler, whereas bill length was measured with a caliper (peak to skull, to the nearest 0.1 mm). Body mass was recorded to the nearest 0.1 g.

The research was approved by the Animal Welfare Committee of Flinders University (permit numbers E312 and E386). Permit to undertake scientific research in SA was granted by SA Department of Environment, Water and Natural resources (permit number Z24699-9). All birds were banded under permit number 2601 from the Australian Bird and Bat Banding Scheme.

To assess whether song complexity or plumage coloration is related to female quality, we used female size and body-condition as a determinant of female intrinsic quality and conducted a principal component analyses on these traits (detailed descriptions are attached in the Supplementary Material). Body condition was determined by using residuals of body mass not explained by size (tarsus length; detailed descriptions are attached in the Supplementary Material).

Only fertile females were included in the analyses to control for variation in singing behavior due to reproductive state. Fertility status was verified according to the following three parameters: (i) development of the brood patch (not fully developed), (ii) nest building status (females were considered fertile until the first egg was laid), and (iii) sexual behavior patterns (copulatory behavior, male display, female solicitation behavior; Mulder, 1992 in Cooney and Cockburn, 1995).

Song Recordings and Analyses

Solo songs of color-banded birds occur naturally between 08:00 and 12:00 h (after the dawn chorus) and were recorded from a distance between 5 and 15 m using a parabolic microphone (Telinga Microphones, Sweden) connected to a portable Sound Devices 722 digital audio recorder (Sound Devices LCC, U.S.A). All sound files were recorded as broadcast wave files (24 bit 48 kHz).

Recordings were transcribed to an Apple MacPro (Apple Corporation, U.S.A) and edited with Amadeus Pro 2.1.2 (Hairersoft Inc, Switzerland). Spectrograms were created using Raven 1.5 on the Hann algorithm display type (filter bandwidth 270 Hz, size 256 samples, time grid overlap 50%, grid resolution 2.67 ms, 188 Hz, DFT 256 samples). Only songs that could be confidently assigned to observed color-banded females were analyzed. In total, 82 songs from 28 females were analyzed. For each song, we measured the total number of elements per song ("song length"), and the number of different elements per song ("song complexity"). We define a song as a complex vocalization composed of several different element types (as described by Langmore and Mulder, 1992), and defined an element in the song as a single, continuous trace on a spectrogram. We categorized the different element types according to previously classified element types (A, F, O, P, Q, R, T, U, V, W) developed by Langmore and Mulder (1992), Colombelli-Négrel et al. (2010), Kleindorfer et al. (2013), and Evans and Kleindorfer (2016), and newly identified element types (FL, H, K, L, Z, ZN). For the analysis we determined the element frequency per song as (i) the total number of elements per song (we refer to this variable as "song length"), and (ii) the number of different element types per song (we refer to this variable as "song complexity").

Spectrometry

We measured the tail coloration of females ($N = 41$), using a JAZ-2000 spectrophotometer and a Xenon-pulsed light source, connected through a bifurcated fiber-optic probe (Ocean Optics, Eerbek, Netherlands). To exclude disturbance by outer light sources and to ensure a standardized distance and angle (90°), a black rubber cylinder was fitted to the top of the probe. Before each measurement the spectrophotometer was recalibrated using a standard white (Avantes, Eerbek, Netherlands); for calibration of black the probe was removed from the light source and the cap of the plug closed (Mahr et al., 2012). Standard descriptors of reflectance spectra were used for quantification of colors. Measurements were taken from five areas on the tail feathers. Calculations were carried out for reflectance in the 320–700 nm range, which is regarded as visual spectrum of most passerine species (Hill and McGraw, 2006). To quantify the UV-reflectance of the blue tail we chose a commonly used variable, namely UV-Chroma (Johnsen et al., 2005; Griggio et al., 2010; Mahr et al., 2012), which is defined as proportion of UV-reflectance on total reflectance (UV:R320–R415/R320–R700; Hill and McGraw, 2006).

Statistical Analyses

To test for the relationships between song length and morphological parameters and plumage characteristics we applied a General Linear Mixed Effects Model (GLMM). Song complexity was analyzed using a Generalized Linear Mixed Effects Model (GZLMM) with a Poisson-distribution as model residuals did not achieve normal distribution even after transformation. Both initial models included UV-Chroma, size, and condition as covariates. As Kangaroo Island and mainland populations are considered to represent different subspecies, we included study site ("location") in all the initial models as a fixed factor to assess local variation in morphology and ornament expression (Dudaniec et al., 2011; Kleindorfer et al., 2013). Also, all the initial models included the interaction between UV-Chroma and location as well as body-size and location because we aimed to test for differences between the populations in regard to UV-Chroma and body-size. Female ID was included as random factor to control for non-independence of multiple measurements from the same female. We had to exclude six females from the analyses since there was not sufficient data available.

The relation between morphological parameters and plumage characteristics was tested separately, due to a difference in the sample size. Analyses were carried out using a General Linear Model (GLM). This GLM included the factor location and the covariates condition, size and the interaction of condition and size. UV-Chroma and condition can show variation during the breeding season, therefore we also included capture month into the GLM. Since the analyses revealed no significant effects of capture date on UV-Chroma and condition, this factor was not included in the GLMM and GZLMM.

We tested for a correlation between song length and complexity using a Spearman's rank correlation test. Song length and complexity were not correlated (Spearman's rank correlation: $\rho = 0.14$, $S = 0.45$, $p = 0.24$), thus we treated these variables independently.

All statistical analyses were performed using "R" (version 2.14.1; R Development Core Team, 2011). We implemented linear mixed effects models using the "lme" function of the "nlme" package. All models were conducted using stepwise forward and backward introduction of terms. Beginning with the interactions, non-significant terms were step by step eliminated from the model. Each eliminated term was re-entered in the final model to obtain statistics (Grafen and Hails, 2002; Engqvist, 2005). In addition to model selection based on *p*-values we performed model averaging using AIC to assess comparability and reliability of both methods. AIC model averaging was implemented using the "model.avg" function of the "MuMIn" package. No differences in the significant results became apparent, and details on the results from model averaging based on AIC-values can be found in the Supplemental Material.

RESULTS

The GLM revealed no significant differences in UV coloration between mainland and island populations [mainland: $N = 18$, island: $N = 23$; $F_{(1,40)} = 2.29$, $p = 0.13$], furthermore no significant effect of month of capture was found on plumage color [$F_{(1,37)} = 0.87, p = 0.46$]. Female size was not related to plumage [$F_{(1,39)} = 0.72, p = 0.40$], and no relation between UV coloration and female condition was detectable [$F_{(1,39)} = 0.18$, $p = 0.67$]. Also, there were no significant interactions between location and body size [$F_{(1,39)} = 0.15$, $p = 0.70$] or month and condition [$F_{(1,39)} = 0.84, p = 0.48$].

Female song length was neither related to her condition [Mainland: $N = 13$, Island: $N = 9$; GLMM: $F_{(1,17)} = 1.93$, $p = 0.18$] nor to her UV-Chroma [GLMM: $F_{(1,19)} = 0.07$, $p = 0.79$]. Also, the interaction of both variables turned out to be non-significant [GLMM: $F_{(1,18)} = 0.06, p = 0.95$]. Interestingly though, we found that study site predicted female song length as females from Kangaroo Island produced significantly longer songs compared to females from mainland populations [GLMM: $F_{(1,20)} = 10.79, p < 0.01$; **Figure 1**]. Also, we found a significant interaction effect of study site and female size on song length [GLMM: $F_{(1,20)} = 5.66$, $p = 0.03$]: Larger females produce longer songs compared to smaller ones, though this effect is only evident on Kangaroo Island (see **Figure 2**). We found no significant main effect of female size on song length [GLMM: $F_{(1,20)} = 0.81, p = 0.38$].

In contrast to song length, song complexity did not differ significantly between study sites (mainland: $N = 13$, island: $N = 9$; GZLMM: $\beta \pm SE = -0.19 \pm 0.11$, $z = -1.69$, $p = 0.09$), though this effect was only marginally non-significant. Female size (GZLMM: $\beta \pm SE = -0.08 \pm 0.05$, $z = 1.58$, $p = 0.11$) and the interaction between female size and study site (GZLMM: $\beta \pm SE = 0.03 \pm 0.13, z = 0.21, p = 0.83$) turned out to have no significant relationship with song complexity. Also, female condition (GZLMM: $\beta \pm SE = -0.003 \pm 0.01, z = -0.26$, $p = 0.79$), UV-Chroma (GZLMM: $\beta \pm SE = -0.80 \pm 1.04$, $z = -0.77$, $p = 0.44$) and the interaction between UV-Chroma and

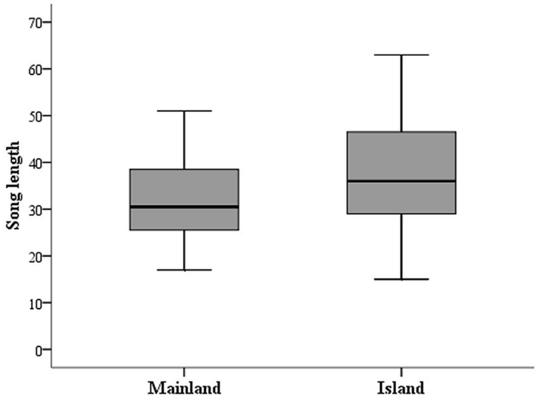

FIGURE 1 | Boxplot of female song length originating from Mainland or Island populations.

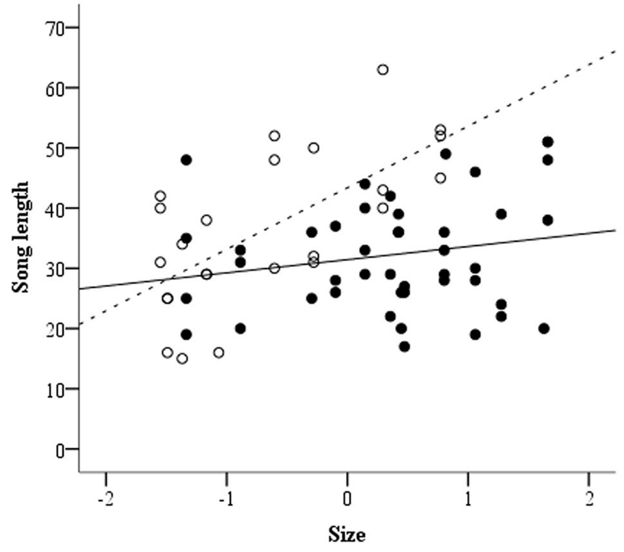

FIGURE 2 | Relationship between female size and song length in mainland (black circles and solid line; $R^2 = 0.04$) and island (white circles and dashed line; $R^2 = 0.44$) populations.

study site (GZLMM: $\beta \pm SE = 1.44 \pm 3.37$, $z = 0.42$, $p = 0.67$) showed no significant relationship with song complexity.

DISCUSSION

Our results show no relationship between plumage ornamentation and song characteristics in female Superb Fairy-wrens, but we revealed a positive relationship between the song length (total number of elements females produce per song) and body-size in females of the Kangaroo Island sub-species. Thus, female Superb Fairy-wrens that sing songs composed of more elements are bigger than females with shorter songs.

In our study populations, the average number of elements varies dramatically between females (between 16 and 50 elements per song). Some females produce more than twice the number of elements than others, which reveals strong individual differences in song strophe length. Consequently for fertile female Superb Fairy-wrens song length may possibly act as a signal for conspecifics to indicate quality. Our results also suggest that songs produced by females of the Kangaroo Island subspecies are significantly longer. These results are in line with previous findings by Kleindorfer et al. (2013), possibly indicating selection processes favoring the production of longer songs within the island population.

Hence, given that size can be an indicator of condition during early development, our results imply that song might act as an honest signal and underlies sexual selection processes in female Superb Fairy-wrens. The idea that female song signals individual quality is supported by earlier studies in Superb Fairy-wrens and New Zealand bellbirds (*Anthornis melanura*), indicating that female song performance (song rate and song complexity) predicts reproductive success (Cain et al., 2015; Brunton et al., 2016).

There are several explanations for why song length in female Superb Fairy-wrens could be an honest signal (Martin-Vivaldi et al., 1998; Farrell et al., 2012; Ferrer et al., 2015). First the production of longer songs is energetically demanding and requires certain physiological preconditions, since it forces females into a trade-off between allocating energy resources toward singing or other activities (Gil and Gahr, 2002). Secondly, the primary function of female song in Superb Fairy-wrens is suggested to be resource defense (Cooney and Cockburn, 1995; Cain and Langmore, 2015). In this context song length might be an indicator for the ability of an individual to defend resources. Finally, very recent findings (Kleindorfer et al., 2016), support the idea, that singing behavior in female Superb Fairy-wrens can also be costly in terms of increased nest-predation. Even though the study by Kleindorfer et al. (2016) refers to song rate rather than song length, one might expect that females producing longer songs may also face higher predation risk by exposing themselves toward predators.

Interestingly, the relationship between size and song length only applies to females from the Kangaroo Island subspecies, but not to females from the mainland populations. However, due to the low sample size, this result has to be treated with caution. A possible explanation for this result could be that Superb Fairy-wrens are in general considered to be long-lived and maintain long-term territories over several years. Stable territories like on the mainland may imply a reduced necessity of intense territorial behavior. In contrast some Superb Fairy-wren populations on Kangaroo Island have been affected by severe bushfires in 2007 (Peace et al., 2011). Within the last years the population started to recover and the number of breeding pairs is increasing in this region. One might assume that individuals face increasing competition from new intruders. Therefore, more competitive individuals, with the ability to maintain larger territories and therefore more resources, should be favored by selection processes. Given that song can be perceived over longer distance and indicates body-size, female song length might signal competitive abilities toward neighbors and intruders (Searcy et al., 2008). Therefore, singing behavior might primarily be of importance to continuously communicate dominance and prevent actual intrusion. Given that, due to natural reestablishment of breeding populations, Kangaroo Island birds might face more frequent encounters with intruders and investment into signals indicating quality might be beneficial to retain breeding sites. Furthermore, this idea is in line with previous findings from Cooney and Cockburn (1995), who demonstrated that female song-rate increased when territories were newly established.

Female song length might also serve as a quality indicator for male conspecifics (Amundsen, 2000). Even though clutch size might not be affected by body size, as females lay a maximum of three eggs per clutch in our population (own observation), choosing bigger females might provide other direct and indirect benefits to males. Size parameters can affect performance in foraging and territorial defense and, as previously mentioned, size can act as indicator for better condition during early development and might signal good genetic quality (Johnson, 1987; Amundsen et al., 1997; Amundsen, 2000). However, given that Superb Fairy-wrens are known to have the highest number of extra pair fertilizations within passerines (Double et al., 1997) and song acts as a signal over long distances, females might also signal quality to possible extra-pair mating partners. The recruitment of extra-pair fertilizations might in turn enhance female reproductive success by increasing genetic variability in the offspring (Andersson, 1994).

Our analyses further reveal that song complexity and song length are not correlated, raising the question of whether both song features signal different quality traits and carry multiple signaling functions. However, song complexity, which has been shown to be an important male song feature for many species (Gil and Gahr, 2002), seems to play only a minor role for female Superb Fairy-wrens. In our study, female song complexity does not reflect female condition or size, nor, in contrast to song length, varies significantly between populations, which has already been shown by previous studies (Dudaniec et al., 2011; Kleindorfer et al., 2013). Furthermore, female song complexity is not related to UV-reflectance of the tail feathers. Also the low variation in song complexity (between three and eight different elements) in comparison to the average number of elements between individual females points toward an inferior role in sexual selection. In previous studies it has been suggested that in some species male repertoire size (e.g., number of elements or songs males produce) does not predict pairing success and therefore plays a minor role in selection processes (Catchpole, 1986; Gil and Gahr, 2002; Byers and Kroodsma, 2009). This might also apply to female song complexity in Superb Fairy-wrens. Nevertheless, it has to be considered that female song complexity reflects quality parameters not recorded in this study.

Whereas a relationship between ornamental features and song performance has been found in interspecific comparisons for female songbirds (Garamszegi et al., 2007; Webb et al., 2016), our results indicate that there is no relationship between UV-Chroma and song features in female Superb Fairy-wrens. This indicates that both traits have evolved independently, rather than co-evolved.

However, it has to be considered that both studies did not examine relationships between song performance and plumage coloration within populations and Garamszegi et al. (2007) focused on carotenoid based plumage features (Garamszegi et al., 2007; Webb et al., 2016). Unlike carotenoid based coloration, UV-coloration is due to melanin based coloration and keratin structure (Prum, 2006). Since these two types of plumage ornaments underlie different physiological mechanisms, they may underlie different selection processes.

UV-Chroma is known to reflect condition of individuals, since the maintenance of UV-reflectance is time consuming and might further result in a trade-off between preening and e.g., parental activities or vigilance behavior (Redpath, 1988; Cucco and Malacarne, 1997; Shawkey et al., 2003). In this context, we expected a correlation between UV-Chroma and condition, but this particular prediction was not supported by our results. One possible explanation might be that the UV-coloration of the plumage is determined by the condition during molt, as shown in male Superb Fairy-wrens (Mulder and Magrath, 1994).

In summary, this is one of the first studies investigating multiple signals in a female songbird, suggesting that plumage features and song performance might underlie different selection processes. Our study revealed that song length is related to a trait reflecting quality and supports the idea of song as a sexually selected trait in female passerines (Cain et al., 2015; Kleindorfer et al., 2016). Our data provides new information on female song and plumage ornaments and more importantly, it extends our understanding of singing behavior in female songbirds.

AUTHOR CONTRIBUTIONS

KM, HH, and MG designed the study; KM and CE conducted the field work and the data collection; KM, HH, KT, and CE performed the statistical analyses of the data; KM, HH, and MG wrote the manuscript with contributions from KT and CE.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: http://journal.frontiersin.org/article/10.3389/fevo.2016.00043

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Evolution of courtship display in Estrildid finches: dance in relation to female song and plumage ornamentation

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Estrildid finches are known for great interspecific diversity in the degree of elaboration in courtship dance, song and plumage coloration and also for the considerable sex differences in these traits within species. To study the evolution of multimodal sexual signaling in these taxa, we collected data on 85 species and analyzed them in a phylogenetic comparative study. As dances are often displayed in parallel with songs and include behavioral elements to emphasize coloration, they are likely to evolve non-independently of songs and plumage characteristics. Hence, we hypothesized that the degree of elaboration in dance would be associated with song and plumage ornamentation due to correlated responses between traits. Alternatively, each sexual signal may evolve independently of the others under the influence of different aspects of reproductive ecology or life history. Finally, because trait expression occurs in both males and females and can be caused by pleiotropic effects, we predicted correlated exaggerations of the traits between sexes as well. We found that courtship dance, song and plumage coloration evolve independently, as these traits were not correlated among species in either sex (i.e., the presence of female song was not related to female dance repertoire). However, we found evidence for correlated responses between the sexes, as species that have males with complex dances or colorful plumage also have females with exaggerated traits. Yet, selection factors acting on these traits were only partially shared between sexes. While coloration can be predicted by intraspecific brood parasitism and dance by body size in females, we were unable to reveal similar relationships in males. Our results indicate that different secondary sexual characters in Estrildid finches evolved under the influence of complicated selection factors, in which both correlated responses between sexes as well as independent selective mechanisms play roles.

Keywords: courtship display, duet, female dance, female song, monogamous songbird, phylogenetic comparative approach, sexual selection

INTRODUCTION

Theories of sexual selection are classically concerned with the evolution of secondary sexual characters that are expressed by males (Andersson, 1994). Textbook examples usually focus on song displays and plumage coloration in passerine birds (Searcy and Andersson, 1986; Hill, 1991, 2006; Searcy, 1992; Catchpole and Slater, 2008), and a large number of phylogenetic comparative studies have identified the key selection factors that shape the tremendous amount of interspecific variance in these traits (e.g., Read and Weary, 1992; Owens and Hartley, 1998; Badyaev and Hill, 2000; Dunn et al., 2001; Mountjoy and Leger, 2001; Jawor and Breitwisch, 2003; Garamszegi and Møller, 2004). Even though it is known in many animal taxa that females choose mates based on male motor performance (review in Andersson, 1994; Byers et al., 2010), non-vocal behavioral elements of the courtship display of birds, i.e., dances, have attracted relatively less attention in the comparative evolutionary context. A few distantly related taxonomic groups, including bowerbirds (e.g., Patricelli et al., 2002; Coleman et al., 2004), birds of paradise (Pruett-Jones and Pruett-Jones, 1990; Scholes, 2008), and manakins (Prum, 1990, 1998; Bostwick and Prum, 2005; DuVal, 2007; Fusani et al., 2007) are characterized by their spectacular behavioral performance, but generally applicable explanations for how such complex traits evolve are rare (Balmford, 1991; Andersson, 1994; Madden, 2001; Galván, 2008). In addition, hardly any study considered multiple sexual traits within the same study to investigate the independent or correlated evolution of various morphological and behavioral sexual display traits.

The evolution of courtship dance in birds constitutes a case that is especially interesting for at least two reasons. First, dances are rarely performed in isolation from other sexual characters, as they are often displayed in parallel with some vocalization and include behavioral elements to emphasize coloration (e.g., Torres and Velando, 2003; Cooper and Goller, 2004; Dakin and Montgomerie, 2009; Dalziell et al., 2013). Therefore, elaborate dances are likely to evolve non-independently of songs and plumage characteristics. For example, in dabbling ducks, the repertoire size of male courtship display is larger in species with sexually dimorphic plumage color (Johnson, 2000) while across the sexually monochromatic species of Pelecaniformes, the complexity of male advertising display is negatively correlated with the conspicuousness of feather coloration (Galván, 2008).

Second, dances can function as interactive signals between the sexes rather than simply being a secondary sexual character of males. For example, pairs of some monogamous birds, such as the Western grebe (Nuechterlein and Storer, 1982), the blue-footed booby (Torres and Velando, 2003), and several cranes (Masatomi, 1983) jointly show intricate ceremonial behavioral sequences in a well-coordinated or synchronized way. Such mutual displays are typically observed in long-lived monogamous non-passerine birds of large body size with sexes of similar appearance as in the above examples, but are also rarely performed by songbirds. These displays can be interpreted as having similar functions to vocal duets, as they can contribute to pair formation, pair bonding, or mate guarding (Malacarne et al., 1991; Wachtmeister, 2001). Although avian vocal duets have attracted much attention (review in Hall, 2004, 2009), the evolution of non-vocal, mutual display behaviors is more veiled in mystery. Furthermore, it is a puzzling question how song and dance displays can simultaneously function and evolve as intersexual communication signals.

In general, the evolution of multimodal sexual signaling can be characterized by the combination of both independent and correlated mechanisms acting on traits and on the sexes (**Figure 1**). For example, sexual selection can promote the evolution of multiple sexual characters, in which multiple ornaments are favored to better convey multiple messages about male quality (Møller and Pomiankowski, 1993; Candolin, 2003), or in which an evolutionary tradeoff between different sexual signals introduces negative interspecific associations between these traits (Badyaev et al., 2002; Shutler, 2011). Accordingly, the former mechanism would predict that the evolution of different sexual traits is under the influence of different selection regimes, which in turn would allow these traits to evolve rather independently of each other (**Figure 1, arrow** [**4**]). However, if different signals indicate the same aspect of quality or are underpinned by the same tradeoffs, different traits would be responsive to the same selection factors that would lead to the correlated evolution of these traits (**Figure 1, arrow** [**3**]). Furthermore, displays that are shared between males and females raise the question of whether these displays evolve independently or as a correlated response of the sexes. For example, mutual mate preferences promote equally exaggerated sexual signals in males and females especially in biparental species (Amundsen, 2000), while duet signaling (e.g., singing or dancing) can also contribute to pair bonding or mate guarding (Malacarne et al., 1991; Wachtmeister, 2001; Hall, 2004, 2009), both of which are expected to cause a correlated evolution of traits as a response to the same selection factors arising from reproductive ecology and/or life history. Furthermore, different traits in different sexes can be under the control of the same pleiotropic mechanisms, e.g., hormones, genes or neurotransmitters, which would couple the two traits on an evolutionary time scale (**Figure 1, arrow** [**1**]) and cause similar expressions of the same trait in both sexes (**Figure 1, arrow** [**2**]) (cf. Ketterson et al., 2005; Møller et al., 2005; but also see Garamszegi, 2014; Goymann and Wingfield, 2014).

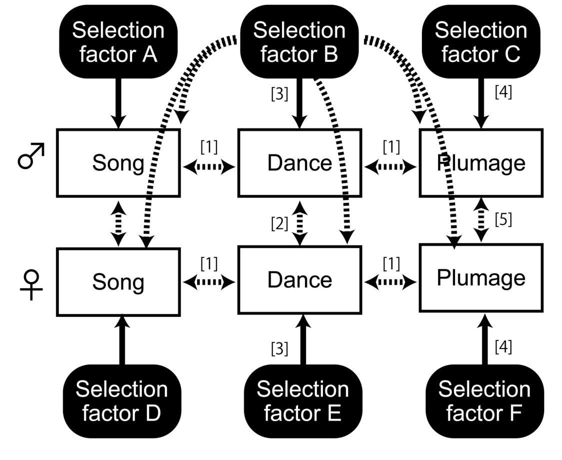

FIGURE 1 | Schematic view of the possible evolution of three sexual signals in males and females under multiple selection pressures. One-headed arrows show selection as exerted by an ecological/life history trait, and two-headed arrows show correlated responses. Solid and dashed lines show known or potential effects respectively. For illustrative purposes, the multimodal effects of particular selection pressures are only emphasized for a single factor (Selection factor B). Similar mechanisms may also apply to the other factors.

Courtship behaviors of Estrildid finches (order: Passeriformes, family: Estrildidae) represent an ideal model for investigating the correlated evolution of audio-visual signaling. Interestingly, virtually all species are socially monogamous (Goodwin, 1982; Payne, 2010), and most of them are sedentary with only a few of them making local movements (Payne, 2010). Migration and deviations from social monogamy have been suggested to be associated with the loss of duetting and female song (Price et al., 2009; Logue and Hall, 2014) and of ornamental plumage coloration (Friedman et al., 2009). Although these driving mechanisms apparently vary little in Estrildid finches, they show great interspecific diversities in the degree of elaboration in courtship dance, presence or absence of female courtship song and dance (i.e., duet or solo performance), and also in plumage color. Furthermore, many species can be characterized by a considerable intersexual dimorphism in these sexual traits. To our knowledge, all Estrildid finches have males that show both dance and song, while females of some species do not dance or sing (Goodwin, 1982; Baptista et al., 1999; Payne, 2010). Given the monogamous breeding biology and the mutual performance of dance between sexes in some species, one can hypothesize that elaborate courtships play important roles in forming and confirming pair bonds rather than in female choice in these finches. However, the degree to which such coordinating mechanisms explain the vast heterogeneity in sexual dimorphisms in song and dance remains unknown. Understanding the evolutionary significance of dance in Estrildids is challenging, as most passerines sing without dance displays.

Our goal in this study was to investigate, by using phylogenetic comparative approaches, the evolution of mutual courtship dance in addition to their complex songs and bright plumages in Estrildids, and what selection factors shape interspecific variation in the complexity of dance. Specifically, to examine correlated evolutionary responses between traits, we tested if courtship dance displays in Estrildid finches show co-evolutionary exaggeration or tradeoff with other sexual displays (**Figure 1**). Accordingly, we predicted that interspecific variations in the presence and complexity of song displays would be associated with interspecific variations in dance displays. If songs and dances were favored for the same function, such as to advertise concordantly the same aspects of mate quality or parental investments, these selection pressures would derive positive correlations across species. Alternatively, if tradeoff mechanisms constrain the evolution of these traits, we can expect a negative interspecific association to occur. In addition, to better understand the evolutionary origins and trajectories of song and dance, we used ancestral state reconstructions and tested for phylogenetic signal. We also predicted the correlated evolution of plumage coloration and behavioral (i.e., song and dance) displays. To investigate correlated evolutionary responses between sexes, we also explored the potential effects of some characteristics of breeding ecology and life-history on the evolution of complex courtship dance acting either concordantly or separately on males and females. As a consequence of correlated evolutionary responses between sexes, we predicted a positive relationship to be detected between male and female dance repertoires, or other sexual display characters, across species. However, if independent selection mechanisms also apply, a certain proportion of the interspecific variance in dance complexity in one sex cannot be explained by interspecific variance in dance complexity in the other sex, but should be attributed to some predictor variables.

MATERIALS AND METHODS

We collected data on courtship dance, song, plumage coloration and several life-history traits of Estrildid finches from published literature. Because many Estrildids, including the well-studied zebra and Bengalese finches (*Taeniopygia guttata* and *Lonchura striata* var. *domestica*), are popular among aviculturists, their breeding behaviors have been documented in detail (Goodwin, 1982; Restall, 1996; Payne, 2010).

COURTSHIP DANCE AND SONG

We compiled information on dance behaviors from "Estrildid finches of the world" (Goodwin, 1982), as this source provides detailed descriptions on the behavioral sequences of social displays including courtships as well as their contextual background. The information provided by this source is reliable because it matches with other published research featuring courtship behaviors of some species (Baptista et al., 1999; Zanollo et al., 2013) and also with our direct observation of several other species (M. Soma pers. obs.). Generally in Estrildids, when a male and a female approach each other, courtship is initiated by either of the sexes, more often by males, which is sometimes followed by interactive phases in which both sexes show the same behaviors, e.g., hopping or bowing, in coordinated ways. The dance is typically terminated by females performing copulation solicitation displays that often result in copulation (Goodwin, 1982; Restall, 1996; see also **Movie S1**, **S2**). Females of some Estrildid species do not dance at all, or show only a portion of males' body movement repertoires.

Their courtship dance is stereotyped within species, and expressed as a combination of several simple actions that can be assigned into the following nine categories: (1) bobbing, i.e., up and down movement of head; (2) horizontal body movement, such as turning or changing body angle or pivoting; (3) moving wing; (4) tail movement, such as angled or spread tail; (5) erection of feathers on body; (6) hopping or stepping; (7) holding nest material in the bill; (8) bill wiping, i.e., rubbing the bill on the perch; and (9) opening the mouth or showing the tongue. Assuming that the repertoire size reflects the degree of elaboration of displays, we counted how many dance categories constitute the courtship displays of each species, separately for both males and females. We found that the repertoire of female dance is always included in the male's repertoire. When we focused on female dance repertoire size, we created statistical models in which male dance repertoire was included as a predictor variable (i.e., we avoided calculating the ratio of sex-specific repertoire owing to statistical reasons).

Unfortunately, song complexity could not be characterized in detail due to lack of published information in many species. Therefore, we scored singing behaviors for each species by first confirming male song, and then determining whether or not the female had songs (scored as 1–0), based on published descriptions in Goodwin (1982), Restall (1996) and Payne (2010).

RESEARCH EFFORT

The available information on presence of female dance and songs and repertoire of dance behaviors may be biased by the intensity of studies targeting the species (cf. Garamszegi et al., 2007). Intensively studied species may appear to have a richer dance repertoire simply because an increased research effort likely reveals rare dance elements. To control for such potential bias, we estimated research effort by counting the number of publications resulting from a keyword-based search using species' names in Web of Science, as done in other studies (Nunn et al., 2003; Lefebvre et al., 2004; Garamszegi and Møller, 2012). Estimates of research effort were log 10 transformed.

PLUMAGE COLORATION

Plumage coloration was scored by measuring the coverage of ornamental plumage coloration in each sex relying on the color plates in Payne (2010). In particular, we considered black, red, and blue/purple as these are considered major ornamental colorations in birds (e.g., Keyser and Hill, 1999; Badyaev and Hill, 2000; Jawor and Breitwisch, 2003). However, we did not apply spectrophotometric techniques, and so invisible colors such as UV could not be considered. To measure the coverage of ornamental plumage, we divided body surface into 21 plumage regions, which were belly, breast, chin, cheek, crissum, crown, flank, forehead, greater primary coverts, greater secondary coverts, lore, lesser wing coverts, mantle, median wing coverts, nape, primaries, rectrices, rump, secondaries, throat, and upper tail coverts (Andersson and Prager, 2006). We scored each region as 0: not colored, 0.5: partly colored, or 1: all colored, and summed these scores across bodies. So, for example, if a bird is all black, it is given a score 21. In this way, we considered the coverage but could not differentiate color types (i.e., black, red, or blue/purple) because Estrildid finches show great among-species variation in plumage color types that are assumed to play roles in sexual contexts (Goodwin, 1982).

LIFE-HISTORY TRAITS

Information on life-history of species was compiled mainly from Payne (2010) except when otherwise stated. We considered variables that likely affect the cost of reproduction, such as incubation period in days, median clutch size, and body size taken as length. We have subsequently excluded incubation period from the set of explanatory variables to avoid multicolinearity, as our preliminary analysis revealed that it strongly correlates with body size. Estrildid finches show considerable among-species variation at least in three aspects, which can reflect differences in reproductive investment or resource competition among conspecifics. First, as some Estrildid finches in Africa are targets of interspecific brood parasitism by birds belonging to the genus Vidua (Sorenson et al., 2004), the presence or absence of interspecific brood parasitism was scored as 0–1. Second, some Estrildid finches show intraspecific brood parasitism (Yom-Tov, 2001), which was scored as 0–1. Third, although most species do not defend territories, the degree of gregariousness varies among species. To score this phenomenon, species were ranked as follows: colonial breeders or species with social systems in which multiple pairs keep contact with each other even during breeding season (3); highly social and gregarious but mainly outside the breeding season (2); breed usually in pairs or in small parties (1); and territorial (0).

COMPARATIVE ANALYSES

We prepared a dataset for phylogenetic comparative analyses that included 85 species, for which we could collect the above behavioral and reproductive variables from the published literature, and for which phylogenetic relationships could be abstracted from http://birdtree.org/ (Jetz et al., 2012). Our biological hypotheses were translated into statistical models that were fitted to the data and incorporated phylogenetic relatedness of species.

We first examined whether dance complexity in each sex is associated with the other sexual displays (i.e., song and plumage) and dance complexity in the other sex. Accordingly, we built a statistical model with male dance repertoire as the response variable and male plumage ornamentation as well as the presence of female song as predictor variables (**Figure 1, arrow [1]**) while controlling for phylogeny and research effort. In this model, we included female song to reflect the strength of selection factors acting on vocalization, because males of each species sing causing no interspecific variation in the presence of male song. In an analogous model for females, female dance repertoire was the response variable, and the predictor variables also included male dance repertoire to test for signs of correlated evolution between sexes (**Figure 1, arrow [2]**). Second, to investigate which life-history variables vary with exaggerated dance displays in males and females, we built statistical models for these sex-specific variables, in which we entered body size, clutch size, coloniality, presence of inter- and intraspecific parasitisms, and research effort as predictor variables (**Figure 1, arrow [3]**). Again, to account for correlated responses between sexes, models on female dance also involved male dance repertoire as a predictor variable (**Figure 1, arrow [2]**). Additionally, we repeated the above-mentioned analyses also for plumage coloration of each sex to investigate correlated responses between behavioral and plumage ornamentation traits, while controlling for phylogeny and research effort. Specifically, we tested for a relationship between plumage coloration and the presence of female song and dance complexity in each sex (**Figure 1, arrow [1]**), and also analyzed if interspecific variance in life-history variables can predict interspecific variance in plumage ornamentation (**Figure 1, arrow [4]**). To make inferences about the correlated responses between sexes (**Figure 1, arrow [5]**), models on female plumage coloration considered male plumage coloration among the list of predictor variables. Due to the poor information that were available for song displays, and because males do not show any interspecific variance in the presence of song, we did not created analogs models for the vocal display traits (i.e., only used the presence of female song as response variable in the other models above).

Non-independence of data owing to the phylogenetic relatedness of species was controlled by using phylogenetic generalized least-square (PGLS) regression technique with maximum-likelihood model fitting with a variance-covariance matrix of the data based on phylogeny with the maximum-likelihood value of λ (the strength of the phylogenetic signal). For these analyses, we could not obtain an overwhelmingly supported single phylogenetic tree with branch lengths, but we could derive multiple and equally likely candidate trees from Jetz et al. (2012). Hence, to account for such phylogenetic uncertainty, we fitted models on each of them and applied multi-model inference (Garamszegi and Mundry, 2014). Specifically, we used 1000 alternative trees from the global birdtree database for our list of species to estimate the phylogenetic parameters of interest (i.e., regression slopes) based on identical model definition in terms of the list of predictor and response variables. Then we derived mean and confidence estimates for these parameters over the pool of phylogenies via model averaging, in which parameter estimates from each model were weighted according to its relative fit to the data. We therefore present model-averaged regression slopes, SE and 95% confidence intervals for the explanatory variables (Garamszegi and Mundry, 2014). These exercises were done using R 3.0.2 (R Core Team, 2013), relying on packages "caper" (Orme, 2012), and "AICcmodavg" (Mazerolle, 2013) and following the example codes on http://www.mpcm-evolution.org/practice/online-practical-material-chapter-12. We opted to use multimodel inference over the pool of alternative phylogenetic trees based on information theoretic approaches instead of adopting Bayesian approaches to deal with phylogenetic uncertainty, because the latter introduces a considerable uncertainty in parameter estimates when non-informative priors are used (Garamszegi and Mundry, 2014).

Before interpreting the model outcomes, to avoid misleading results based on statistical artifacts, we performed numerous model diagnostic statistics on a PGLS model that incorporated the consensus phylogeny obtained from the birdtree sample of trees. We first checked assumptions about the distribution of

Table 1 | Associations among three sexual signals in Estrildid finches.

Explanatory variable	Coefficient	SE	95% CI
(A) MALE DANCE REPERTOIRE			
Intercept	4.484	0.460	[3.582 to 5.386]
Male plumage coloration	−0.001	0.036	[−0.073 to 0.071]
Presence of female song	−0.206	0.464	[−1.115 to 0.702]
Log research effort	**0.617**	**0.249**	**[0.129** to **1.105]**
(B) FEMALE DANCE REPERTOIRE			
Intercept	−1.112	1.007	[−3.087 to 0.862]
Male dance repertoire	**0.537**	**0.137**	**[0.270** to **0.805]**
Female plumage coloration	0.004	0.047	[−0.087 to 0.095]
Presence of female song	0.546	0.621	[−0.670 to 1.763]
Log research effort	**0.713**	**0.301**	**[0.123** to **1.303]**
(C) MALE PLUMAGE COLORATION			
Intercept	7.609	1.958	[3.771 to 11.447]
Male dance repertoire	−0.057	0.337	[−0.717 to 0.604]
Presence of female song	2.424	1.432	[−0.382 to 5.230]
Log research effort	−0.478	0.780	[−2.007 to 1.050]
(D) FEMALE PLUMAGE COLORATION			
Intercept	0.869	0.628	[−0.362 to 2.100]
Male plumage coloration	**0.684**	**0.057**	**[0.571** to **0.796]**
Female dance repertoire	−0.052	0.116	[−0.280 to 0.176]
Presence of female song	−1.128	0.694	[−2.487 to 0.232]
Log research effort	−0.207	0.407	[−1.004 to 0.591]

*The relationship between plumage coloration and female song and dance repertoires in both males **(A)** and females **(B)** while controlling for research effort. In a similar way, we also tested if dance complexity and the presence of female song is associated with plumage coloration in the two sexes [males **(C)** and females **(D)**]. Each coefficient was estimated from model averaging over 1000 phylogenetic trees. Bold typeface is used when 95% CI does not contain zero, thus can be interpreted as significant effect.*

residuals i.e., whether they were normally and homogeneously distributed. The visual inspection of the corresponding diagnostics plots (e.g., Q-Q plot and residuals plotted against fitted values) indicated no obvious deviations from these assumptions. Second, we examined issues about multicollinearity that might potentially lead to instable results and unreliable parameter estimates (Freckleton, 2011; Mundry, 2014). For this purpose, we calculated variance inflation factors (VIF, O'Brien, 2007) to the standard linear model analog of each phylogenetic model that was obtained after excluding the phylogenetic component (as the meaning of VIF is not obvious within the PGLS framework). After the exclusion of incubation period, these analyses showed that collinearity among predictors is not a serious issue to consider further (VIFs < 2). Finally, we verified the absence of influential data points by excluding each of them one by one from the data and then contrasting the derived parameter estimates and fitted values against those that correspond to the model based on the full data. This jackknife procedure revealed no evidence for influential cases strongly affecting the interpretations of the model outcomes.

To characterize the evolutionary history of different sexual traits in different sexes in more detail, we performed ancestral state reconstructions using Mesquite (Maddison and Maddison, 2011) and R package "phytools" (Revell, 2012). To compare the phylogenetic constrains acting on female dance and song, under the prediction that underlying mechanisms that govern trait expressions are shared, we calculated phylogenetic signals λ using R package "phytools" (Revell, 2012). In these analyses, we relied on 58 species with a completely bifurcating phylogenetic tree with branch lengths as reported in Arnaiz-Villena et al. (2009). We adopted maximum-likelihood analyses as it has several advantages over the method based on parsimony (Cunningham et al., 1998; Pagel, 1999).

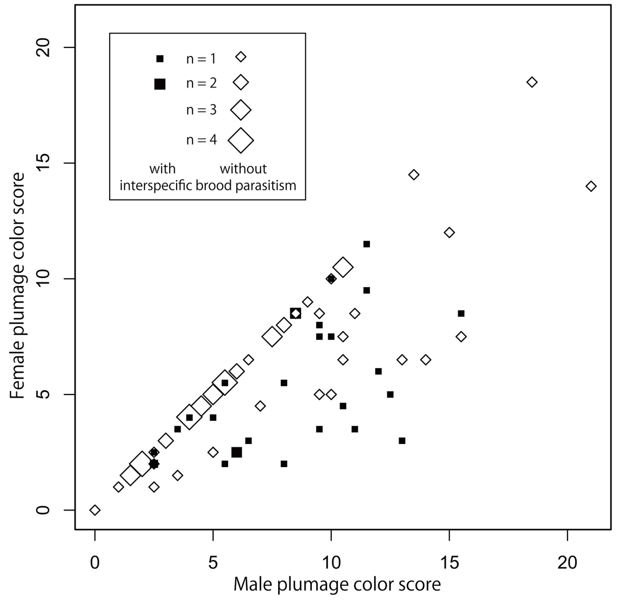

FIGURE 2 | Correlation of plumage coloration between sexes when interspecific brood parasitism is also considered (see Table 3). Each dot shows the scores of the coverage of ornamentally colored (i.e., red, black, and blue/purple) plumage areas in males and females of each species. Filled square dots stand for the species with intersecific parasitism (n = 27) while open dots for those without it (n = 58).

RESULTS

CORRELATED RESPONSES BETWEEN TRAITS AND SEXES

Overall, we found that dance complexity of Estrildid finches was not associated interspecifically with the presence of colorful plumage or females song (**Tables 1A,B**). We also found no correlation between plumage coloration and the other sexual traits (**Tables 1C,D**). However, we found that females had complex dance in those species in which males also had complex dance (**Table 1B**), and that females were more colorful in species with colorful males (**Table 1D**), suggesting correlated responses between sexes (**Figure 2**). Although we found evidence for research effort confounding estimates of sexual difference in dance complexity, such effects do not confound the focal relationships (**Table 1**).

The common ancestor of Estrildid finches is likely to have female dance (**Figure 3**), but its dance complexity might have been lower for females compared to males (**Figure 4**). The weak, if any, relationship between female song and dance (**Table 1**)

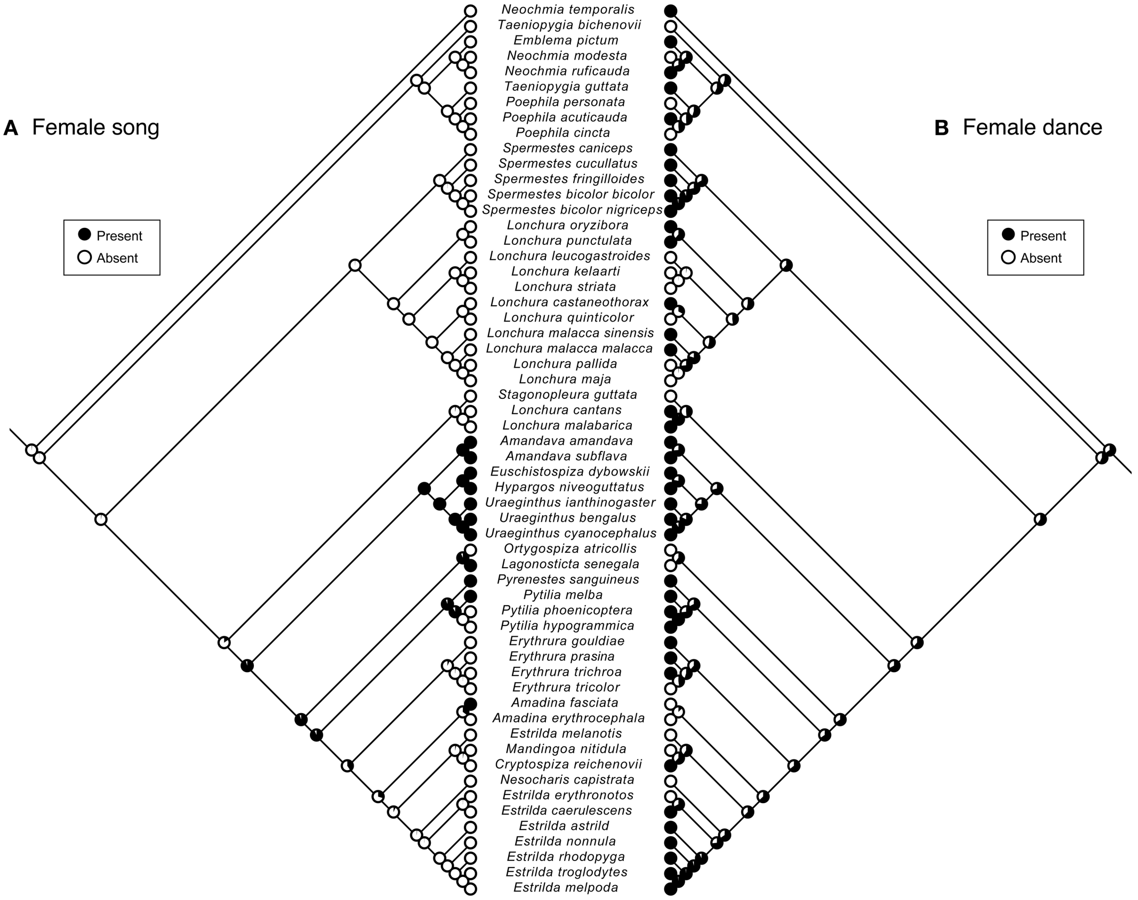

FIGURE 3 | Ancestral reconstruction of behavioral sexual signals in females. Maximum-likelihood reconstruction of ancestral states for the evolution of female song **(A)** and dance **(B)** in Estrildid finches, as shown along the phylogeny.

is also apparent when traits are plotted along the branches of the underlying evolutionary tree as binary traits (**Figure 3**). While phylogenetic signals for presence of female song were strong and statistically significant ($\lambda > 0.99$, $P < 0.0001$), those of female dance was not ($\lambda = 0.47$, $P = 0.81$), indicating that related species tend to be similar with respect to the presence/absence of female song but not dance. This result further indicates that female dance and song followed different evolutionary trajectories. In addition, the common ancestor of Estrildid finches is likely to lack female song but not female dance (**Figure 3**).

INDEPENDENT EVOLUTION OF FEMALE DANCE AND PLUMAGE COLORATION: THE ROLE OF LIFE-HISTORY TRAITS

We detected that partially different factors could be responsible for the evolution of dance complexity in males and females (**Table 2**). While in the PGLS model for males, none of the candidate predictors showed significant associations, in females, dance complexity was positively related to body size when male dance complexity was held constant (**Table 2**, **Figure 5**). Furthermore, there was a marginal tendency for intraspecific parasitism to affect dance complexity in both sexes (**Table 2**).

We also found that different factors might be responsible for the evolution of plumage coloration in males and females (**Table 3**). While none of the candidate predictors showed strong relationships with male coloration, the parallel analysis for females indicated that this sex had less colorful areas on their plumage under higher risk of brood parasitism (**Table 3**, **Figure 2**).

DISCUSSION

EVOLUTION OF MULTIMODAL SEXUAL SIGNALS

The evolution of multimodal sexual signals is a topical issue in today's evolutionary biology. This series of phylogenetic comparative analyses in the monogamous Estrildid finches indicated that visual and vocal courtship displays evolved independently (**Figure 6**), in sharp contrast to the prediction that song and dance constitute a complementary sexual signal. The presence of female song was unrelated to both dance repertoire size and coloration. Although dance complexity was correlated between sexes, the underlying factors that contribute to the exaggeration of dance complexity were only partially shared between males and females. In addition, we also found similar roles for plumage coloration. Plumage coloration was not correlated with the other

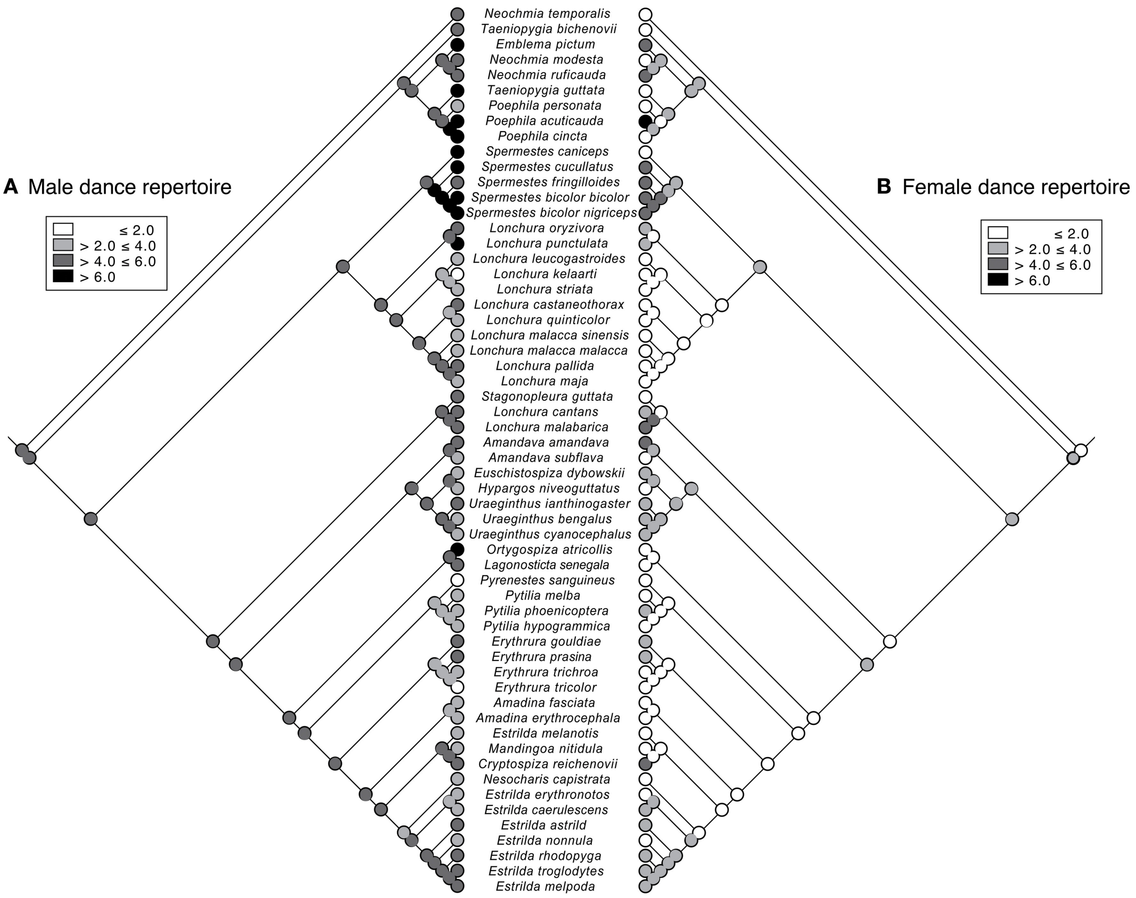

FIGURE 4 | Ancestral reconstruction of dance repertoire. Maximum-likelihood reconstruction of ancestral states for the evolution of dance repertoire size in male **(A)** and female **(B)** in Estrildid finches, as shown along the phylogeny.

sexual signals, but was correlated between sexes. These findings suggest that courtship dance and its mutuality between sexes, and plumage coloration in Estrildids evolved under the influence of complicated selection factors, in which both correlated responses between sexes as well as independent selective mechanisms play roles.

Functions of multi-modal displays can be explained in two ways: either multiple displays (e.g., audio-visual displays) are redundant and communicate the same information efficiently, or they convey different messages (Møller and Pomiankowski, 1993; Johnstone, 1996; Candolin, 2003; Bro-Jørgensen, 2010). Our results in association with dance, song, and plumage characters in Estrildid finches support the latter because these three sexual traits were evolutionarily independent of each other. The strong phylogenetic signal for the presence of female song in this groups suggests its evolution is more constrained – perhaps due to the neuroendocrinological and morphological adaptations usually associated with birds song (Bolhuis et al., 2010). Forces of sexual selection that favor ornamental plumage coloration and song do not explain interspecific variation in dance complexity of either sex. This finding indicates that independent selective mechanisms should be considered for explaining the emergence and maintenance of spectacular courtship dance displays in some passerine bird species. Apparently, different mechanisms may be in effect at the within- and between-species level, as it has been reported in males of one Estrildid species (diamond firetail, *Stagonopleura guttata*) that the quality of courtship dance (i.e., bobbing speed) and plumage ornamentation were positively correlated (Zanollo et al., 2013). Clearly this result is not applicable to the among-species context.

SELECTION FACTORS FOR CORRELATED EVOLUTION BETWEEN SEXES

One important selection factor that could account for the maintenance of complex display traits is mutual sexual selection to enhance synchronization between pair members. Mirroring behaviors, such as showing similar gestures, or mimicking vocalizations, can be a form of affiliative communication and can contribute to the formation and maintenance of social bonds in a range of animal taxa (e.g., call convergence in budgerigars: Hile et al., 2000; vocal duets in pairs of gibbons; Geissmann and Orgeldinger, 2000; contagious yawning in dogs: Romero et al., 2013), that can be particularly important in many bird species with song duets (Langmore, 1998; Amundsen, 2000; Hall, 2004). Although we did not quantitatively assess the degree of coordination/synchronization of dance displays between sexes, in Estrildid finches, the repertoire of female visual displays is

Table 2 | Effects of life-history traits on dance complexity in male (A) and female (B) Estrildid finches.

Explanatory variable	Coefficient	SE	95% CI
(A) MALE DANCE REPERTOIRE			
Intercept	4.143	1.806	[0.602 to 7.683]
Body size	0.035	0.115	[−0.190 to 0.260]
Clutch size	−0.083	0.242	[−0.558 to 0.392]
Coloniality	0.090	0.222	[−0.344 to 0.525]
Interspecific brood parasitism	0.518	0.404	[−0.274 to 1.311]
Intraspecific brood parasitism	1.248	0.679	[−0.083 to 2.579]
Log research effort	0.257	0.332	[−0.393 to 0.908]
(B) FEMALE DANCE REPERTOIRE			
Intercept	−5.910	2.448	[−10.708 to −1.111]
Male dance repertoire	**0.480**	**0.146**	**[0.194** to **0.766]**
Body size	**0.441**	**0.156**	**[0.136** to **0.746]**
Clutch size	0.015	0.310	[−0.593 to 0.622]
Coloniality	0.062	0.281	[−0.490 to 0.613]
Interspecific brood parasitism	0.665	0.559	[−0.431 to 1.760]
Intraspecific brood parasitism	1.737	0.902	[−0.031 to 3.504]
Log research effort	0.187	0.414	[−0.624 to 0.998]

Each coefficient was estimated from model averaging over 1000 phylogenetic trees. Bold typeface is used when 95% CI does not contain zero, thus can be interpreted as significant effect.

Table 3 | Effects of life-history traits on plumage coloration in male (A) and female (B) Estrildid finches.

Explanatory variable	Coefficient	SE	95% CI
(A) MALE PLUMAGE COLORATION			
Intercept	8.035	5.648	[−3.034 to 19.104]
Body size	0.009	0.359	[−0.694 to 0.712]
Clutch size	0.185	0.768	[−1.321 to 0.392]
Coloniality	−0.715	0.690	[−2.066 to 0.637]
Interspecific brood parasitism	−0.329	1.273	[−2.824 to 2.166]
Intraspecific brood parasitism	−1.259	2.091	[−5.358 to 2.839]
Log research effort	0.357	1.022	[−1.645 to 2.360]
(B) FEMALE PLUMAGE COLORATION			
Intercept	1.097	2.837	[−4.463 to 6.658]
Male plumage coloration	**0.665**	**0.056**	**[0.555** to **0.775]**
Body size	0.067	0.172	[−0.270 to 0.403]
Clutch size	−0.190	0.390	[−0.955 to 0.574]
Coloniality	0.095	0.351	[−0.592 to 0.783]
Interspecific brood parasitism	**−1.274**	**0.598**	**[−2.447** to **0.102]**
Intraspecific brood parasitism	−0.673	1.057	[−2.745 to 1.399]
Log research effort	−0.178	0.524	[−1.206 to 0.850]

Each coefficient was estimated from model averaging over 1000 phylogenetic trees. Bold typeface is used when 95% CI does not contain zero, thus can be interpreted as significant effect.

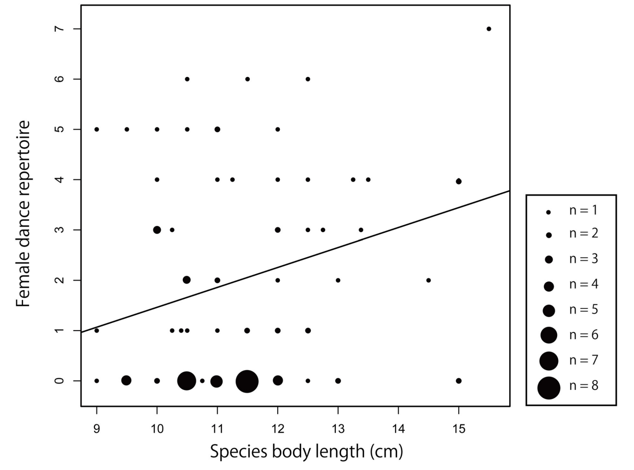

FIGURE 5 | Interspecific association between body size and dance complexity of females. Dots are species-specific estimates, line is the PGLS regression line that is estimated in the corresponding phylogenetic model (**Table 2**).

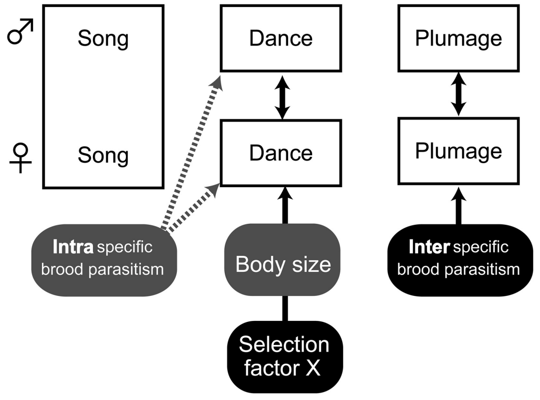

FIGURE 6 | Schematic view of the results of the analyses on the evolution of three sexual signals. Two headed arrows show correlated evolution between sexes, and one-headed arrows show factors responsible for trait evolution. Marginal effects are shown with dashed arrows. The figure summarizes the results from **Tables 1–3**.

covered by that of males without exception. At least some species have been shown to exhibit behavioral synchronization that contributes to better breeding success of pairs (Adkins-Regan and Tomaszycki, 2007; Elie et al., 2010; Mariette and Griffith, 2012; see also **Movie S1**), and male and female dance repertoire depict correlated evolutionary responses. Therefore, one can reasonably infer that interspecific variation in dance complexity is likely resulted from selection pressures for behavioral coordination between the sexes. However, further studies are needed about the importance of such coordinated dance displays between sexes.

We also found that male dance repertoire is consistently larger than that of females and that the phylogenetic signal for the presence of female dance compared with female song is weaker. These findings suggest that species can gain or lose female dance traits under less phylogenetic constrains. Females of some species may have lost the ability to dance similarly to males, and under relaxed selection pressures behavioral sexual dimorphism (in terms of smaller dance complexity in females than in males) may arise owing to reduced dance complexity in females. The emergence of sexual dimorphism in dance repertoires may suggest that in addition to correlated evolutionary responses between sexes, female dance complexity may follow partially independent evolutionary routes from that of male dance complexity. Such scenario would be analogous to the findings of comparative studies on female song showing that songbirds might have common ancestors with female song, which was lost in some lineages (Garamszegi et al., 2007; Odom et al., 2014). Which selection factors caused some Estrildid species to lose ancestral dance ability while others have maintained it during their long evolutionary history? Such a question was investigated in a phylogenetic comparative study of New World blackbirds, which appeared to have ancestors with female songs and socially monogamous breeding systems, but in some lineages female song was lost owing to changes in life-history such as the emergence of polygyny (Price, 2009). However, Estrildid finches lack drastic variations in mating systems as all species are socially monogamous, which makes the evolution of courtship dances a different story, and subject to further investigation.

Another selection factor that can explain the evolution of complex sexual signals in both sexes is mutual mate preferences. Female ornamentation can evolve when males are choosy, and both sexes can be equally ornamented when mate choice is bidirectional (Amundsen, 2000). This explanation fits to the evolution of plumage coloration in Estrildid finches as females have more ornamental colors in species having colorful males (**Figure 2**). Moreover, it is also possible that female song and dance evolved similarly in response to male mate choice. However, it is clear that mutual mate choice is not a major common factor, given the observed independent evolution of multiple sexual signals.

LIFE HISTORY TRAIT AND SEXUAL SIGNALS

Some life-history traits of Estrildid finches that we considered in this study could be candidate factors responsible for the evolution of duet singing and/or dancing, but our result showed that, except body size, none of them can explain interspecific variation in dance complexity (**Table 2**). The only significant pattern we detected indicates that some proportion of the interspecific variation in dance complexity in females can be accompanied by a parallel variation in body size (**Table 2B**). Since body size is associated with numerous life-history traits (Bennett and Owens, 2002), based on our correlative results, it remains difficult to elucidate which correlates of body size is associated with the evolution of courtship dance. A marginally significant tendency for intraspecific parasitism also emerged indicating that dance complexity in both sexes might be related to the degree of cuckoldry within-species. If such relationships were proven to be true, it would suggest that mutual dance evolved in response to increased reproductive cost, a mechanism that would be similar to what has been proposed for duet singing (Hall, 2004). Accordingly, well-coordinated behaviors assured by mutual signaling could lead to better reproductive success when both pair members need to invest more into reproduction when facing with a higher risk of intraspecific brood parasitism. Alternatively, well-coordinated mutual dance displays could signal pair-bonding to others, which would in turn reduce the risk of becoming the target of intraspecific brood parasitism.

Another interesting finding on the evolution of sexual signals in Estrildid finches was that the coverage of ornamental plumage coloration in females decreased when interspecific brood parasitism was present (**Table 3B**). This suggests that having more cryptic appearance should be advantageous in terms of having lower chances to be detected by brood parasitic birds that search host nests. Analogous results were reported for species that are parasitized by the brown-headed cowbird *Molothrus ater*, indicating that males of host species tended to have less conspicuous sexual signals, i.e., songs under higher parasite pressure (Garamszegi and Avilés, 2005). Given that Estrildid females generally stay longer in the nest during nest-building period as compared to males (Payne, 2010), such selection pressures particularly acting on females might serve as a likely explanation for the results we obtained in this study.

Overall, this study highlights the similarity between mutual dance display in Estrildid finches and duet song performance in other songbirds (Hall, 2004), which would contribute to our understanding of functional significance of female dance in Estrildid finches. Moreover, our approach has revealed that studying sex-specific characters in males and females instead of sexual dichromatisms leads to better understanding of the dynamic evolution of sexual traits. However, it remains unclear why some female Estrildid finches sing as surprisingly little is known about vocal communication in this taxonomic group. In contrast with a few limited Estrildid species without females song (e.g., Zebra and Bengalese finches) that have been well studied, singing behaviors of males and females of the rest of the species are largely uncertain (but see Gahr and Güttinger, 1986; Geberzahn and Gahr, 2011, 2013; Kagawa and Soma, 2013; Ota and Soma, 2014), and can be a crucial key to understand the evolution of multimodal communication in future studies.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: http://www.frontiersin.org/journal/10.3389/fevo.2015.00004/abstract

Movie S1 | Mutual courtship display of the Java sparrow.

Movie S2 | Male courtship display of the red-cheeked cordon-bleu.

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VOCAL INTERACTIONS BETWEEN FEMALES AND MALES

Females vocalise with males to form duets in Peruvian warbling-antbirds.
Photo: Joe Tobias

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Duetting as a Collective Behavior

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Mated birds of many species vocalize together, producing duets. Duetting behavior occurs at two levels of organization: the individual level and the pair level. Individuals initiate vocalizations, answer their mates' vocalizations, and control the structure and timing of their own vocalizations. Pairs produce duets that vary with respect to duration, temporal coordination, and phrase-type combinations, among other properties. To make sense of this hierarchical structure, organize duetting research, and identify new avenues of investigation, we advocate a "collective behavior" approach to the study of duets. We critically review key terminology in the duetting literature in light of this approach, and elucidate six insights that emerge from the collective behavior approach: (1) Individual-level behaviors describe pair-level behaviors, but the opposite is not true; (2) The level of organization informs how we test for the rules that govern behavior; (3) Functional hypotheses about duetting must distinguish individual from group characters; (4) Stimulus-response, cybernetics, and entrainment offer alternative hypotheses for the cognitive control of duetting behavior; (5) Avian duetting has the potential to be a model system for the ontogeny of vocal interaction; and (6) The collective behavior approach suggests new avenues of research. Ultimately, we argue that nearly every aspect of duetting research stands to benefit from adopting a collective behavior approach. This approach also has applications to other forms of interactive vocal communication in birds and primates, including humans.

Keywords: animal communication, chorus, conversation, countersinging, cybernetics, causality, female song, levels of organization

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INTRODUCTION

In hundreds of species of birds, mated pairs sing duets (Hall, 2004, 2009). The black-bellied wren (*Pheugopedius fasciatoventris*) is one such species. Researchers might take one of two approaches to describe its duetting behavior. They might say that both sexes initiate song and both answer their mates' songs to form duets (Logue and Gammon, 2004). They might also note that the mates' prior phrase-type influences both the answerer's phrase-type and, in the case of females, the latency to answer. In short, they could describe *what the individuals do* during the act of duetting. Alternatively, the researchers might quantify the proportion of duets initiated by each pair member, the repertoire of duets that each pair can produce, or the amount of overlap between male and female phrases. That is, the researchers could describe *what the pair does* during the act of duetting.

As this example illustrates, duetting occurs at two levels of organization: The individual level and the pair level. The properties of duets (e.g., duet-type, duet duration) are pair-level characters because they arise from the integration of individual phenotypes. Song initiation and song answering are the individual-level behaviors that generate duets, so variation in duet structure is caused by variation in initiation and answering. Important dimensions of variation in song initiation include the decision to initiate and the choice of phrase-type. Dimensions of variation in answering include the decision to answer, the choice of answer phrase-type, and the fine-scale timing of the answer.

We can codify the relationship between the levels of behavior by saying that duetting is a collective behavior that arises from the interaction of two individual-level behaviors, song initiation and song answering. The term "collective behavior" describes behavior at the group level, like murmurations of starlings (*Sturnus vulgaris*; King and Sumpter, 2012), fish shoals (Couzin and Krause, 2003), or huddling rat pups (Schank and Alberts, 1997). Collective behavior can arise from local interactions (Couzin, 2009), where local can refer to both space and time. As such, functional behaviors can emerge at the group level without any global blueprint. Research on collective behavior often involves large collectives of individuals that follow simple rules, resulting in group-level behaviors that would be difficult to predict based on the individual-level behaviors (Goldstein, 1999; Couzin, 2009). In contrast, duets are produced by small collectives of individuals, resulting in group-level behaviors that are not necessarily difficult to predict based on the individual-level behaviors. Neither of these differences, however, prohibit the application of the collective behavior approach to duetting. To the contrary, as the simplest possible groups, two-individual collectives are tractable test cases that can inform collective behavior research.

Our thesis is that it is critical to acknowledge that duetting occurs at both the individual and pair levels. This idea has ample precedent in the literature. Although early work implicitly assumed that pair mates' interests were perfectly aligned (Hall, 2004), the 1980s and 1990s saw a turn toward an individual-level approach to functional research on duetting (Wickler, 1980; Sonnenschein and Reyer, 1983; Levin, 1996a,b). In the 2000s, Logue (2006) argued that an improved understanding of the individual-level behaviors that structure duets would "elucidate previously unconsidered functional links and operational constraints" (pp. 326–327). Shortly thereafter, Brumm and Slater (2007) described duetting as "collective signaling," and hypothesized that functionality might emerge at the pair level. In her influential review of vocal duetting, Hall (2009) summarized the state of the collective behavior approach and called for its broad application: "Distinguishing between pair-level aspects of duetting and the underlying individual behaviors . . . is essential to understanding how and why duetting occurs." (pp. 67, 69). Here, we clarify and expand on these ideas.

The utility of the collective behavior approach should be evaluated in comparison to the alternative approach, which is to ignore the distinction between individual-level and group-level behavior. Acknowledging that duetting occurs at two levels offers two kinds of benefits:

1. It provides a logical framework to guide duetting research. Research on duetting is complicated by the fact there are two possible levels of organization. The collective behavior approach forces researchers to specify the level of interest, encouraging precise definitions of problems, questions, and research objectives and reducing ambiguity. For example, many papers address the "function of duetting." That term could refer to the function of initiation, answering, or both behaviors for the male, the female, or both pair members. The collective behavior paradigm requires a specification of the level of organization, eliminating this kind of ambiguity.
2. It suggests new avenues for research. Distinguishing between the levels of organization reveals areas in need of additional research. Moreover, the collective behavior approach encourages work on the relationship between individual-level behavior and the properties of group-level phenomena, like duets (Brumm and Slater, 2007). Finally, this approach facilitates adoption of the theoretical and empirical toolkits that have been developed to study collective behavior.

A comprehensive review of the duetting literature according to this framework would be largely redundant with reviews by Hall (2004, 2009) and Dahlin and Benedict (2014). Instead, we offer a critical review of duet terminology (**Box 1**), and six insights that can be gained by treating duetting as a collective behavior. Collectively, these insights demonstrate that nearly every aspect of duetting research stands to benefit from the collective behavior approach. This report is part of a special issue on female song in birds, so it is worthwhile to articulate its relevance to the study of female song *per se*. Female song is often given in the context of vocal duets (Langmore, 1998), so the biology of female song is intimately related to that of duetting. We invoke the collective behavior approach in an attempt to clarify this relationship.

INSIGHT 1: INDIVIDUAL-LEVEL BEHAVIORS DESCRIBE PAIR-LEVEL BEHAVIORS, BUT THE OPPOSITE IS NOT TRUE

Description is the first phase of most research programs on duetting systems. Here, we consider description in light of the collective behavior framework.

Initiation and Answering

Answering (as defined in **Box 1**) is necessary and, along with initiation, sufficient to generate a duet. After the initiation, all subsequent phrases by either partner in the duet are answers. In some cases, it is useful to distinguish between the initial answer and "secondary answers" that prolong the duet.

The individual that initiates a vocalization does not directly control whether it will be answered, so rates of duet initiation (as in Illes, 2015) are pair-level properties (Hall, 2009). The term "initiation" describes an individual-level behavior but, perhaps counter-intuitively, "solo singing" is a pair-level property because the mate must refrain from answering for an initiation to be a solo song. Thus, the relative rates of duets and of solo songs (Grafe and Bitz, 2004; Mennill and Vehrencamp, 2005; Mennill, 2006) are pair-level properties. So too is the duration of the duet train (Logue, 2007b), which emerges from the answering

BOX 1 | A critical review of terminology describing duets and duetting behavior.

Answer: A vocalization in response to, and in temporal coordination with, a vocalization produced by the duetting partner (Wickler and Sonnenschein, 1989; Riegert et al., 2004; Hall, 2009; Dahlin and Benedict, 2014). Initiation and answering are the individual-level behaviors that are necessary and sufficient to generate duets (Hall, 2009). Answer is synonymous with "reply" (Diamond and Terbourgh, 1968; Mann et al., 2003), but the former term is more common in the contemporary literature. The individual that answers is the "answerer" or "responder."

Answering rule: Individual-level psychological property linking the duetting partners' previous vocalization(s) to the structure of the focal individual's answer (Hall, 2009). Some previous definitions also included rules governing phrase timing (Logue, 2006, 2007b; Logue et al., 2008), which we now treat separately (see "Timing rule").

Call: A vocalization that is not a song. Many species duet using calls.

Contribution: All parts of a duet produced by one individual (e.g., "the male's contribution comprised three phrases").

Coordination: The regularity of the temporal relationship between the male and female duet contributions. Coordination can be measured at the individual level and at the pair level.

Duet: A pair-level vocalization comprising one initiation and one or more answers. In nearly all duetting bird species, duet partners are pair-mates. Notable exceptions are the male-male duets in cooperatively displaying manakins of the genus *Chiroxiphia* (Trainer et al., 2002).

Duet code: A set of answering rules that links the phrase-type a focal individual hears to the phrase-type that the focal individual uses to answer (Logue and Gammon, 2004; Logue, 2006, 2007b). A duet code is an individual-level property, but some or all of the links comprising a code may be shared between partners or among other individuals. To answer according to a duet code is to "adhere" to a duet code (Logue and Gammon, 2004). The term "song fitting" is synonymous with adhering to a code (Rogers et al., 2006).

Duet train: A duet that includes two or more answers (e.g., male-female-male; Brown and Lemon, 1979).

Duet repertoire: In species with duet-types, the set of types that a pair or population produces is its "duet repertoire" or "duet-type repertoire" (Levin, 1996a; Hall, 2009).

Duet-type: A structurally distinct duet. Duet-type is a pair-level property that is generated by individual-level answering rules. Duet-type is a useful term for species with highly stereotyped duets (e.g., Sonnenschein and Reyer, 1983; Rogers, 2005).

Gap: A silent interval between the phrases of a duet. Gaps can be measured at both the individual level (e.g., the female's average latency relative to the end of the prior male phrase) and the pair level (e.g., the total gap duration over a whole duet).

Initiation: A vocalization that is not an answer. Initiations constitute the beginning of a duet if the partner answers, otherwise they are solo vocalizations (songs or calls). Initiation is an individual-level behavior (e.g., "the male initiated 13.2 vocalizations per hour"). The sex that initiates a duet, however, is a pair-level property (e.g., "the pair produced 3.2 male-initiated duets per hour"; Grafe and Bitz, 2004; Hall, 2004; Logue and Gammon, 2004).

Non-random phrase-type combination: A statistical association between the phrase-types that partners use in duets. Non-random phrase-type combinations at the level of the whole duet are pair-level phenomena. Adherence to a duet code by one or both sexes is one of several ways that pairs can generate non-random phrase-type combinations (Logue, 2006, 2007b).

Overlapping: The simultaneous production of sound by both duetting partners. Overlap can be measured at both the individual level and the pair level. Duets in which there is no overlap between subsequent phrases are sometimes described as "alternating" (Hall, 2009).

Phrase: The minimum vocal unit that an individual contributes to a duet (Mann et al., 2003). Phrases are structurally identical to solo songs in many species. The term "phrase" is less likely to cause confusion than alternatives, such as "song" (*sensu* Levin et al., 1996), which can also describe whole duets or solo songs.

Phrase repertoire: The set of phrase-types that can be produced by an individual, a pair, or a population. Phrase repertoire should not to be confused with "duet repertoires" (as in Voigt et al., 2006).

Phrase-type: A structurally distinctive class of phrases (Mann et al., 2003; Hall, 2009).

Song: A vocalization involved in mate attraction and territory ownership. Songs are usually louder and longer than calls, and usually exhibit a strong annual and diel rhythm (Catchpole and Slater, 2003). Songs are used as duet phrases in many species. In the duetting literature, the term "song" has been used to mean a solo song, a phrase, and a whole duet. To avoid confusion, we suggest that authors use modifiers to specify their meaning (e.g., "male solo song") when using the term with respect to duet-singing birds.

Termination: The final phrase of a duet, or the act of singing that phrase. Duet termination is a pair-level property. For example, consider the duet "male-female-male." The male terminates because the female does not answer his second phrase.

Timing rule: Individual-level psychological property linking the timing of one or more previous vocalizations to the timing of the focal individual's answer.

behavior of both partners. The preceding statements assume that solo vocalizations are potential duet initiations. Researchers should test this assumption, to avoid lumping classes of solo vocalizations that are never answered with those that are. Care should also be taken to distinguish phrases that are never used in duets from those that change structure in response to being answered (Tobias and Seddon, 2009).

The proportion of duets initiated by males and females are widely reported pair-level statistics in the duetting literature (Gill et al., 2005; Mennill and Vehrencamp, 2005; Hall, 2006; Mennill, 2006; Topp and Mennill, 2008; Mann et al., 2009; Elie et al., 2010; van den Heuvel et al., 2014). Hall (2009, p. 77) warned that "the percentage of duets initiated by one sex or the other is not a good measure of the propensity of the opposite sex to duet, as it does not consider the unanswered (solo) songs of either sex and probably just reflects sex differences in song initiation rates." We agree with the first part of this statement, but further note that differences in duet initiation rates could arise from differences in the individual-level behaviors initiation rate, answer rate, or a combination of the two (**Table 1**).

Many properties of duets depend on both partners' song initiation and song answering behaviors. These include the occurrence of duets, duet rate, duet initiation rates (including the proportion of duets initiated by males and females), duet termination rates, duet train duration, solo singing rates (if solo songs are potential duet initiations), the relative rates of duetting and solo singing, and the proportion of songs that occur in duets.

The individual-level properties, male and female initiation rates and answer rates, can be used to unambiguously reconstruct

TABLE 1 | A sample of individual-level property sets that produce an equivalent percentage of male initiated duets.

Individual-level properties				Pair-level property
Male initiation rate (songs/h)	Female initiation rate (songs/h)	Male answer rate (%)	Female answer rate (%)	Duets that are male initiated (%)
10	10	10	10	50
50	10	50	10	50
10	50	10	50	50
100	100	50	50	50

TABLE 2 | Phrase-type sequences representing five duets from one mated pair of black-bellied wrens (*Pheugopedius fasciatoventris*).

Duet	Phrase sequence					
126	F2	M2	F3	M1		
134	M1	F1	M1	F1	F1	M1
138	M1	F1				
146	F4	M3	F4	M3	F3	M1
147	M3	F4	M3			

F1–F4 are female phrase-types, M1–M3 are male phrase-types. Variation in duration and phrase sequence precludes the assignment of discrete duet-types in this species.

pair-level properties. Pair-level attributes, however, cannot be used to unambiguously reconstruct individual-level behavior. For example, based on the statement "50% of duets were initiated by the male" it is impossible to estimate the magnitude of the individual-level attributes "initiation rate" and "answer rate" or their relative values in the two sexes (**Table 1**). Because of this ambiguity, individual-level behaviors should constitute the main descriptors of duetting behavior (Hall, 2009). Benefits of this approach include clarity (these metrics leave no ambiguity at either level of organization), simplicity (only four numbers are required to describe patterns of initiation and answering, more if secondary answer rates are included) and comparability (metrics can be compared across studies).

Our recommendation to focus on individual-level descriptors of initiation and answering runs counter to some other recommendations for duet description, which include both individual- and pair-level descriptions (Farabaugh, 1982; Dahlin and Benedict, 2014). Indeed, pair-level descriptors should be reported if they are hypothesized to be a salient aspect of the signal (e.g., if extra-pair receivers are known to be indifferent to the individual-level processes that generate duets). Of course, pair-level metrics are the only option in systems where it is impossible to distinguish the partners' duet contributions (Benedict and McEntee, 2009; Benedict, 2010). In such systems, authors should report pair-level metrics and explain their limitations.

Phrase-Type Choice

Analogous to "song-type," the term "duet-type" describes a discrete structural class of duets. Individual patterns of answering and phrase-type choice determine this pair-level property. Duet-types may be shared among all pairs, or distinctive to each pair. When duet-types are shared, pairs may "match" the duets of neighboring pairs, much like individual birds match song-types (Grafe and Bitz, 2004; Marshall-Ball and Slater, 2004).

Duet-type is a useful construct for species with discrete duet structures (Sonnenschein and Reyer, 1983; Rogers, 2005). In many duetting species, however, the exact sequence of phrase-types comprising a duet varies a great deal, prohibiting the identification of discrete types (Vencl and Soucek, 1976; Brown and Farabaugh, 1991; Logue, 2007b; Dahlin and Benedict, 2014; **Table 2**). For example, duets in wrens (Troglodytidae) are characterized by variation in the individual that initiates the duet, the number of answers in the duet trains, and the pattern of phrase-type switching within duets (Marshall-Ball and Slater, 2004). Such patterns are to be expected if duet organization is driven by local, individual-level processes (like adherence to duet codes) rather than global, pair-level processes (like shared, whole duet templates). If duets do not belong to structurally discrete types, pair-level descriptors like "duet repertoire" and "duet sharing" fall short of the desired level of precision. In such cases, we suggest that researchers interested in characterizing variation in duets phrase combinations focus on individual-level properties that influence duet structure, such as answering rules, and pair-level properties that characterize and constrain duetting, such as sharing of answering rules and phrase-types between mates, and the pair's phrase-type repertoire.

Fine-Scale Timing

Most pair-level properties that depend on the interaction of the duetting individuals' timing rules fall under the umbrella of "temporal coordination" (Farabaugh, 1982; Levin, 1996a; Hall, 2009; Dahlin and Benedict, 2014). Coordination can be characterized with respect to phrase overlapping, silent gaps between phrases and variation in these properties (Elie et al., 2010).

In many duetting systems, a pair-level description of duet coordination does not unambiguously specify the individual-level behaviors responsible for phrase timing. For example, an antiphonal duet with high temporal coordination (minimal gaps and overlapping sections) can be achieved by both sexes basing the timing of their phrases on the timing of the mate's previous phrase or by one sex adhering to an endogenous repetition rate and the other sex adjusting its timing in response. As above, however, it is possible to unambiguously infer duet properties from individual-level patterns of behavior. We therefore recommend prioritizing descriptions of duet timing at the individual level. Pair-level properties should also be reported when they bear on research hypotheses.

To conclude this section, the way in which duets are described depends on the level of organization. Many descriptions of duetting in the literature (e.g., Mann et al., 2009) and recommendations to describe duets (Farabaugh, 1982; Dahlin and Benedict, 2014) emphasize the (pair-level) structure of the whole duet. Pair-level descriptions alone are rarely adequate for reconstructing the individual-level behaviors that produce duets. Individual-level behaviors, however, can be used to unambiguously reconstruct pair-level phenomena. We therefore recommend that authors report duetting behavior at the individual level when possible. Some pair-level descriptions may also be worth including (e.g., for ease of comparison between studies, for specific research hypotheses), when space permits.

INSIGHT 2: THE LEVEL OF ORGANIZATION INFORMS HOW WE TEST FOR THE RULES THAT GOVERN BEHAVIOR

The collective behavior approach to duetting research emphasizes the relationship between individual-level behavioral rules and the structure of pair-level duets. Researchers have attempted to identify individual-level answering rules with both observational methods (von Helversen and Wickler, 1971; Vencl and Soucek, 1976; Levin, 1996a; Mann et al., 2003; Mennill and Vehrencamp, 2005; Hall, 2006; Rogers et al., 2006; Valderrama et al., 2013), and interactive playback experiments (Logue, 2006, 2007b; Templeton et al., 2013). Analyses of observational data can be classified as "directional" analyses that measure phrase transitions within duets, or "holistic" analyses that measure associations at the level of the whole duet. Directional associations are measured between a focal phrase and some preceding referent (usually the mate's prior phrase). The order of the phrases is taken as evidence of causation (i.e., earlier events are assumed to influence later ones). Holistic associations are measured non-directionally, at the level of the whole duet (Mann et al., 2003; Mennill and Vehrencamp, 2005).

Phrase-type associations at the level of the whole duet are clearly a pair-level property, as evidenced by the single phrase association matrix that can be produced for each pair. Even in directional studies, however, observational methods cannot unambiguously demonstrate the individual-level answering rules that structure duets. This is because they cannot rule out the possibilities that male and female phrase-types are both influenced by some extrinsic cue, that phrase-type choice in one or both sexes is influenced by a non-vocal signal from the pair mate, or that the mates' intrinsic phrase-type switching programs are correlated (Logue, 2006). Thus, failure to find non-random phrase-type associations in an observational study is evidence against answering rules, but positive findings leave open several possible explanations. Playback experiments in which birds answer randomly ordered duet phrase stimuli allow researchers to rule out alternative explanations, so they provide the strongest evidence of the causal effects of the partner's phrases on the actor's phrase-type choice.

Like answering rules, timing rules can also be investigated observationally (Rivera-Cáceres, 2015) or experimentally (Templeton et al., 2013). Observational studies infer timing rules by modeling the delay to answer as a function of prior events in the duet (Logue et al., 2008; Rivera-Cáceres, 2015). The order of events is assumed to be evidence of the direction of influence. The song answering experimental protocol used to test for answering rules can also be used to characterize timing rules (Logue et al., 2008; Templeton et al., 2013).

Like observational studies of phrase choice, observational studies of timing can be directional or holistic. Directional studies, which are much more common, measure the timing of a focal phrase relative to one or more preceding referents. Such studies may be effective at determining individual-level behaviors, as evidenced by the high concordance between the results of directional observational and experimental approaches in a study that used both (Logue et al., 2008). As with studies of phrase choice, however, experiments provide more compelling causal explanations at the individual level than do observational studies. Holistic studies characterize the coordination of the whole duet, but provide little information about individual-level processes.

In conclusion, observational studies are well-suited to characterize pair-level properties of duets, with respect to both structure and timing. We encourage researchers studying the individual-level rules that govern duet participation to adopt the experimental approach.

INSIGHT 3: FUNCTIONAL HYPOTHESES ABOUT DUETTING MUST DISTINGUISH INDIVIDUAL FROM GROUP CHARACTERS

As we have discussed, aspects of a duet can be described as individual phenotypic characters and as group characters. However, it is not always clear which description is relevant to a particular research hypothesis. We suggest that causal thinking can help resolve this issue.

Scientists are chiefly interested in determining the cause of an effect; an association between two variables is rarely considered meaningful unless it sheds light on their causal relationship. In evolutionary biology, a relevant concern is whether a given character caused differences in fitness in the past, giving rise to selection and adaptation. This causal link between character and fitness is the foundation for all questions of evolved function.

Figure 1 formalizes the causal relationships between characters and fitness with path diagrams (Pearl, 2009), in which solid arrows reflect direct causal effects and dotted lines reflect correlations between variables. Consider a focal individual i and her partner j who express, respectively, traits p_i and p_j and, together, a group trait P. There are three possible ways that i's fitness, w_i, may be affected by the expression of these traits: only individual characters directly affect fitness (solid arrows between p_i and w_i and between p_j and w_i, as in **Figure 1A**), only the group character directly affects fitness (solid arrow between P and w_i, as in **Figure 1B**), or both individual and group characters directly affect fitness (solid arrows between p_i and w_i, between p_j and w_i, and between P and w_i, as in **Figure 1C**). According to the logic above, P has functional relevance only in the diagrams in **Figures 1B,C**, because it is only in these diagrams that the group character directly influences fitness. Although **Figure 1A** also contains a relationship between group character and fitness, it is correlational rather than causal.

For example, the decision to initiate a vocalization may influence fitness directly, or it may influence fitness indirectly, via its effects on the group character. At the individual level,

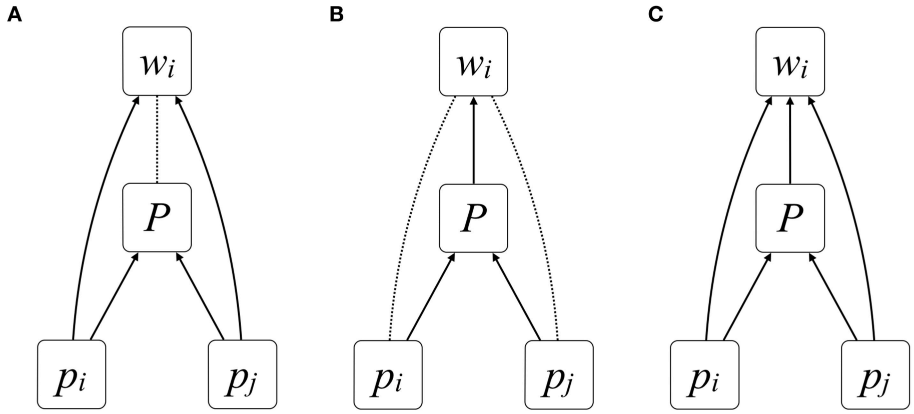

FIGURE 1 | Causation, correlation, and individual vs. group characters. Solid arrows denote direct causal effects of one variable on another, whereas dotted lines denote correlations between them. Fitness, w_i, can be directly affected by **(A)** individual traits p_i and p_j alone, **(B)** the group trait P alone, or **(C)** both individual and group traits p_i, p_j, and P. The group trait P is functionally relevant in **(B,C)** because it has a direct causal effect on fitness and, therefore, selection; it is not relevant in **(A)** because it exerts no influence on fitness.

initiation may have some of the same costs and benefits as solo singing. Indeed, initiation *is* solo singing if the behavior fails to elicit an answer from the partner. However, initiation also affects a group character: pairs with two initiators will duet more frequently than pairs with one initiator; likewise, pairs with one initiator will duet more frequently than those without any initiator. If the frequency of duetting also has a direct causal effect on individual fitness—or, if fitness cannot be measured, on a reasonable proxy thereof—it is a necessary part of the duet "story." Hence, we have two characters under selection: an individual character (the propensity to initiate) and a group one (duet frequency). As in **Figure 1C**, the individual character influences the group character, and both influence fitness.

We offer three clarifications about this argument. First, it is independent of the concept of group selection. Group selection is contingent on direct causal effects of group character on *group fitness*, but group characters can cause changes in either individual or group fitness (Okasha, 2006, 2012; Krupp, in press). This is why **Figure 1** and the example of duet frequency as a causal group character given above are able to make reference only to individual fitness, w_i.

Second, this argument is independent of the concepts of synergy, non-additivity, and non-linearity (Queller, 1984, 1985; Taylor and Maciejewski, 2012; Taylor, 2013). Consider again the example above, relating duet frequency to individual fitness, and let the additional fitness caused by the duet frequency of pairs that have zero, one, or two initiators be, respectively, w_0, w_1, and w_2. If $w_0 = 0$, $w_1 = 1$, and $w_2 = 2$, we would have an additive effect of duet frequency and, if $w_0 = 0$, $w_1 = 1$, and $w_2 = 3$, we would have a synergistic effect. Neither effect, however, has any bearing on the functional relevance of a group character. What matters is contained in the phrase "the fitness effect caused by the duet frequency of pairs," above: that the group character, linear, or otherwise, directly causes differences in fitness (Krupp, in press).

Third, there is no purely statistical method to identify the effect of a group character. This is because causality depends on more than the joint distribution of character and fitness (Pearl, 2009). Group characters depend on individual characters, and so both individual and group characters will predict fitness even though only one may actually cause it (Okasha, 2006, in press; Krupp, in press). Statistical analysis alone cannot determine which of these models describes the true character-fitness causal structure, because each character is connected, either by causation, or by correlation, to fitness.

Signal receivers are major selective agents of signaling behaviors (Owren et al., 2010; Bradbury and Vehrencamp, 2011). A key step toward understanding the function of duetting behavior is figuring out whether receivers attend to individual-level properties, pair-level properties, or both. For example, we might ask "do receivers attend to the overall coordination of a duet or to the fine-scale timing of answers by the duetting individuals?" It is possible to conduct playback experiments to address this question, but we are not aware of any existing studies that attempt to do this with respect to duet timing or any of the other properties discussed above.

The relative salience of individual- and pair-level properties also has implications for the evolution of duetting behavior. If, for example, extra-pair receivers respond to individual-level temporal coordination, selection would drive both sexes toward higher coordination. Alternatively, if the overall temporal coordination is all that matters, one sex might evolve to compensate for the other.

The concept of causality disciplines our thinking about the biological significance of duet-level properties, such as duet duration, duet coordination, and duet-type. At one extreme, if fitness effects are entirely due to individual-level characters, then the duet-level properties of the pair exert no fitness consequences. At the other extreme, duet-level properties may be the only direct causal forces. If this is true, any combination of individual-level behaviors that results in a given duet structure will be functionally equivalent. It seems logical that individual-level functionality could apply to either within-pair or extra-pair receivers, whereas pair-level functionality would tend to apply to communication between the duetting pair and extra-pair receivers. Brumm and Slater (2007, p. R521) touched on the idea of group-level functionality when they wrote, "the interplay between the single components produced by different individuals can form a new quality, which, in turn, may act as a signal itself. This sort of meta-parameter could be formed by the particular phrases that individuals combine . . . or the temporal patterning between the individual contributions of the collective signal." In any given duetting system both individual-level behaviors and duet-level properties may be salient to signal receivers (including the duetting birds themselves). This is, of course, an empirical question. Incorrect assignment of the level of behavioral relevance can distort research (Bell, 2014; Pellis and Pellis, 2015), so care should be taken to test the effects of behavioral variation at both levels.

INSIGHT 4: STIMULUS-RESPONSE, CYBERNETICS, AND ENTRAINMENT OFFER ALTERNATIVE HYPOTHESES FOR THE COGNITIVE CONTROL OF DUETTING BEHAVIOR

Research in collective behavior aims to understand the individual-level decision processes that produce organized group-level phenomena. Duetting birds are ideal systems for research on the cognitive control of group-level behavior because they respond to stimuli immediately, and with high temporal and structural specificity (Thorpe, 1963; Laje and Mindlin, 2003; Amador et al., 2005; Logue, 2007b; Fortune et al., 2011). The relative paucity of studies on the cognitive control of interactive vocal communication in duetting birds contrasts with the large and growing number of such studies in humans and other primates. Drawing from that literature and other approaches to the control of behavior, we lay out three testable hypotheses about the cognitive control of duetting behavior, and describe an approach to link these mechanisms to pair-level duet properties.

Research in Behavioral Ecology generally assumes that the control of behavior can be described by the stimulus-response (S-R) model (Bell, 2014). It is unclear, however, whether S-R accurately models the cognitive control of song answering. A key prediction of the S-R model is that the structure and timing of answers (responses) should depend entirely on the phrase or phrases that are necessary to stimulate the answer (stimuli). The finding that the fine-scale timing of answers depends, in part, on an endogenous rhythm that can be measured in the answerer's previous phrases is evidence against a pure S-R model of the control of song answering (Hall and Magrath, 2007; Logue et al., 2008; Templeton et al., 2013; Rivera-Cáceres, 2015).

Cybernetic theory (Bell, 2014) offers an alternative model for the control of song answering in which individuals attempt to satisfy the "goal state" of hearing a complete phrase pair. For example, suppose that when a female hears Male Phrase X, she pursues the goal state of hearing the phrase pair "Male Phrase X → Female Phrase Y." A distinctive prediction of the cybernetic control hypothesis could be tested by playing the female a stimulus comprising Male Phrase X followed by the first few notes of Female Phrase Y. According to the S-R hypothesis, the female should sing all of Female Phrase Y, but according to the cybernetic hypothesis, she should (at least sometimes) sing only the part of Phrase Y that is not included in the stimulus. One appealing characteristic of the cybernetic model is that it specifies both phrase structure and timing rules, eliminating the need for separate explanations for the evolution of answering rules and timing rules.

A third hypothesis for the cognitive control of duetting behavior sees duet partners as entrained oscillators (Hasson et al., 2012; Takahashi et al., 2013). Hasson et al. (2012) argue that during dyadic vocal interaction, oscillations in acoustic signals entrain with patterns of neural activity within and between interactants. Entrainment might functionally amplify signals and facilitate turn taking. Some existing data are consistent with the hypothesis that the temporal coordination of avian duets results from the coupled oscillator mechanism, as opposed to temporally local call-and-response mechanisms. Answer timing in wrens is influenced by both the focal individual's previous phrases and the mate's phrases, as would be expected given coupling (Logue et al., 2008; Rivera-Cáceres, 2015). Rufous hornero duets offer the best evidence of a coupled oscillator mechanism in birds. As the male hornero's song accelerates, the female locks into a series of rhythmic states relative to the male (Laje and Mindlin, 2003; Amador et al., 2005).

Chief among the methods used to study collective behavior are agent-based models (ABMs; Goldstone and Janssen, 2005). In ABMs, autonomous agents follow behavioral rules triggered by their local environment. These models are well-suited to test hypotheses about the control of answering behaviors. For example, one could program real or virtual duetting robots to follow duetting rules based on S-R, template matching, or entrainment. The ABMs could generate populations of duets, the properties of which could be compared to those of real duets. It would be particularly interesting to see how models behaved over variable inter-individual distances (Logue, 2007a; Templeton et al., 2013) or when more than two individuals answer, as occurs in chorusing species (Mann et al., 2006).

INSIGHT 5. AVIAN DUETTING HAS THE POTENTIAL TO BE A MODEL SYSTEM FOR THE ONTOGENY OF VOCAL INTERACTION

Song learning research has advanced our understanding of word acquisition at the behavioral (Lipkind et al., 2013), neurological (Doupe and Kuhl, 1999), and genetic (Fisher and Scharff, 2009; Pfenning et al., 2014) levels. Although an excellent model of word acquisition, song learning *per se* is not a model for the development of interactive vocal communication because it does not specify the way that individuals use their vocal signals during interactions. An animal model of vocal interaction could promote advances in academic disciplines, like Linguistics and Ethology, as well as applied fields like human-computer interactions and speech pathology. We suggest duetting in songbirds (Order: Passeriformes, Suborder: Passeri) as a model of interactive vocal communication in humans (Todt and Naguib, 2000; Logue and Stivers, 2012). Both processes involve orderly turn taking, both involve listening and rapidly responding with a stimulus-appropriate answer, and both appear to require learning, as we argue below. Unfortunately, we know very little about the ontogeny of duetting behavior, much less its neurological and genetic underpinnings. The collective behavior approach helps to advance this line of research by emphasizing that pair-level development is a consequence of individual-level development on the part of one or both pair mates.

We focus on the ontogeny of duetting behavior during two life phases. Although duetting may develop at other life phases (e.g., during dispersal, while territorial but unmated) existing evidence suggests that early life and the extended pair bonding period (potentially lasting the duration of the pair bond) are particularly important. In the first phase, juvenile birds develop the ability to initiate and answer song, and the rules that structure duets. The second phase of duet development occurs in the context of the developing pair bond. During this phase, individuals may adjust their own duetting behavior in response to the mate's (e.g., to better coordinate, temporally, or structurally, with the mate).

Four lines of observational evidence indicate that juvenile songbirds learn the details of their duetting behavior. First, songbirds learn their songs, including the phrases used in duets (Wickler and Sonnenschein, 1989; Levin et al., 1996; Catchpole and Slater, 2003). Although it is not possible to rule out a scenario in which birds apply non-learned answering rules to learned phrases, parsimony favors the hypothesis that answering rules are also learned. Second, duet codes in many species are individually distinctive (Hall, 2009), but in at least one species same-sex individuals share elements of their codes (Logue, 2006). This pattern of partial code sharing is a key prediction of the hypothesis that codes are learned. Third, juvenile birds have been observed duetting and chorusing with their parents, often mimicking the same-sex parent's role in the duet (Farabaugh, 1983; Brown and Farabaugh, 1991; Rivera-Cáceres and Guerrero Esmeralda, 2014; **Figure 2**). Finally, a recent study of plain wrens offers a comprehensive analysis of the ontogeny of answering rules and timing rules. It shows that juveniles duet with poorer temporal coordination than do adults, answer according to their parents' duet codes, and improve their adherence to that code over time (Rivera-Cáceres and Guerrero Esmeralda, 2014).

Three studies focus on the ontogeny of pair-level duet properties during the extended pair-bonding period. In a cross-sectional study of plain wrens (*Cantorchilus modestus*), pairs that had been together for at least 2 years exhibited stronger associations between male and female phrase-types than did new pairs (Marshall-Ball et al., 2006). Neither Arrowood (1988) nor Benedict (2010) found evidence of changes in temporal coordination over the duration of the pair bond in white-winged parakeets (*Brotogeris versicolurus*) and California towhees (*Pipilo crissalis*), respectively. The pair-level approach to duet ontogeny used by these studies can tell us about developmental changes (or lack thereof) in whole duets that may be salient to receivers. They do not, however, reveal the individual-level ontogenetic processes (e.g., learning) that shape duetting behavior. For example, in the study of plain wrens, it is not possible to determine whether males, females, or both sexes changed their answering rules. Because individuals (rather than pairs) learn, studies aimed at understanding rule learning should focus on the individual level.

Three other studies bear on individual-level duet ontogeny in mated birds. Levin (1996a) conducted a mate exchange experiment on bay wrens (*Cantorchilus nigricapillus*). All four birds in the study answered with more variable timing immediately after re-mating than they did with their previous (established) mate, but only one (a female) met the criterion of statistical significance. The author concluded that "individual repertoires and duet precision do not change following a change in mates" (p. 1093). Our confidence in this conclusion is limited by the study's small sample size (two pairs), the short duration of post-pairing observations (3 days), and the focus on answering rules in males (to the exclusion of females). Logue (2007b) found that female black-bellied wrens adhere strictly to duet codes, whereas males adhere weakly to the reciprocal of their mates' codes. These findings suggest the hypothesis that females enter the pair-bond with a crystallized code, and males learn their mates' codes over time. Hall and Magrath's (2007) cross-sectional analysis of magpie lark (*Grallina cyanoleuca*) duets provides evidence that the fine-scale timing of answers changes over the duration of the pair bond. Both males and females in well-established pairs answered with lower temporal variability than did same-sex birds with new partners (see Figure 2 in Hall and Magrath, 2007).

Thus, the most compelling evidence that answering rules and timing rules develop in mated adult birds comes from cross-sectional studies (Marshall-Ball et al., 2006; Hall and Magrath, 2007). Cross-sectional analyses do not reveal causality, so it is possible that duetting behavior does not develop in mated adults, but rather (a) duet coordination influences pair duration, or (b) some unmeasured factor influences both duet coordination and pair duration.

In conclusion, the ontogeny of duetting behavior has the potential to serve as a model of the development of the rules that govern vocal interaction. Although pair-level research on duet ontogeny can provide biologically relevant information about changes in duet structure, it cannot reveal the development of the individual-level processes that govern duetting. Evidence is accumulating that songbirds learn answering rules and timing rules in early life. We currently lack strong evidence of duet ontogeny in mated individuals. The field stands to benefit from individual-level longitudinal studies of duetting behavior over the course of the pair bond.

INSIGHT 6: THE COLLECTIVE BEHAVIOR APPROACH SUGGESTS NEW AVENUES OF RESEARCH

Explicitly distinguishing the pair and individual levels suggests novel questions about duetting behavior. Here we offer three examples.

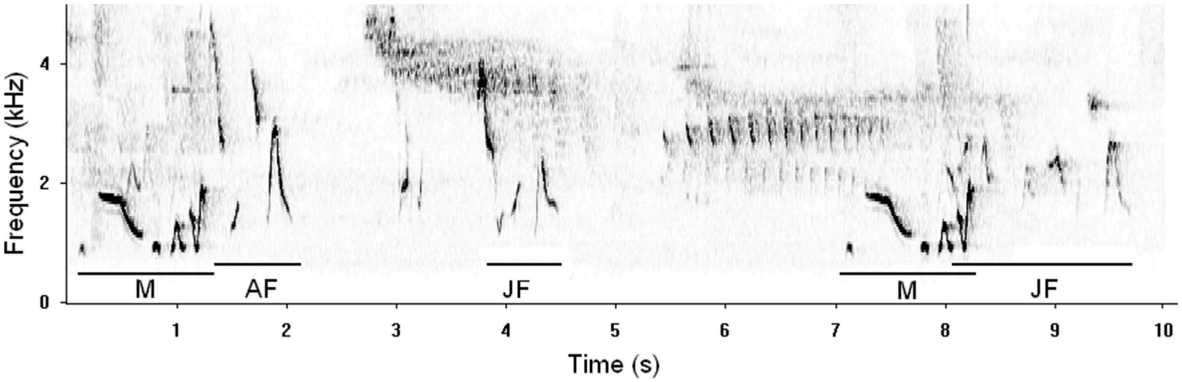

FIGURE 2 | A spectrogram of a field observation that may depict duet code learning. A duet between an adult male (M) and adult female (AF) begins at 0 s. At 4 s the pair's juvenile daughter (JF) approximates the adult female's song. After the male repeats his song at 7 s, the daughter duets with him, again approximating the mother's answer. Spectrograph drawn from a field recording by D.M.L (Gamboa, Republic of Panamá, 2001).

Evolution of Individual-Level Behavior

Several studies adopt the comparative approach to address evolutionary hypotheses about duetting (Farabaugh, 1982; Malacarne et al., 1991; Logue, 2005; Benedict, 2007; Hall, 2009; Mann et al., 2009; Logue and Hall, 2014). All of these studies implicitly focus on the pair-level by categorizing species as either "duetting" or "non-duetting." Although comparative analyses rely on population- or species-level character scores, it is nevertheless possible to score the individual-level traits that characterize each population.

Analysis of the evolution of collective properties at the individual level can reveal evolutionary patterns that are not apparent from group-level analyses (Mank et al., 2005; Price et al., 2009). In the case of duetting, pair-level analyses obscure whether males, females, or both sexes initiate and answer. If answering functions differently in the two sexes (Tobias and Seddon, 2009) or if the cost-benefit ratio of answering differs between the sexes (Logue and Gammon, 2004), lumping the sexes in evolutionary analyses is likely to obscure sex differences in evolutionary patterns of duetting. Further, evolutionary reconstructions at the pair level provide no information about the interaction between answering in the two sexes (e.g., whether the evolution of answering in one sex tends to coincide with the evolution of answering in the other). Comparative studies that treat answering in the sexes separately would remedy this gap in the literature. Analyzing the evolution of initiation rates and answering rates as continuous variables would provide even more information, by addressing the high degree of natural variation in answer rates. Answering is not the only individual-level behavior that has been neglected by comparative studies. No existing studies have looked at the evolution of phrase-type choice or fine-scale timing at the individual level.

Complex and Dynamic Duetting Rules

Duetting rules specify the individual-level decision processes that structure group-level displays. Some species produce complex duets in which each partner produces multiple phrase-types (Vencl and Soucek, 1976; Wickler, 1976; Morton and Gonzalez Alonso, 1982; Brown and Farabaugh, 1991). Others are less complex, but still highly variable with respect to duration and phrase composition (Mann et al., 2003; Logue, 2007b). Yet the simple, first-order duet code is the only experimentally demonstrated answering rule (but see Wright and Dahlin, 2007). In principle, the phrase-type used for answering could be influenced by factors other the mate's previous phrase-type, such as the position of the answer in the duet, or the sequence of prior phrases. It is not clear whether species that produce complex duets abide by complex answering rules, or alternatively, if intrinsic patterns of phrase-type switching combined with simple duet codes explain the structure of their duets. We also know little about how answering rates and answering rules are affected by the answer's position in the duet train.

Although adherence to duet codes is seldom complete (Mann et al., 2003; Mennill and Vehrencamp, 2005; Logue, 2007b), there has not been any published work on the reasons, functional, or otherwise, that adult birds deviate from their duet codes. One possibility is that they simply make mistakes. Another is that codes are more complex than we have realized, so apparent "deviations" are actually defects in the researcher's hypothesized coding rules. A tantalizing speculation is that answering rules include contingencies based on the communicative goal of answering. If answerers choose phrase-types to control the communicative effect of answering, a certain vocal gesture might, for example, request incubation relief or suggest a location for joint foraging. The presence, structure, or timing of answers could communicate the answerers' decision or preference (Flack, 2013).

Answering as a Model of Perception

Individuals' abilities to perceive and distinguish variation in timing and phrase structure influence interactions between duetting individuals, and thus the properties of whole duets. The predictability of rule-based answering suggests the value of duetting birds as models of perception and categorization. Playbacks of manipulated phrase stimuli could identify the structures birds require to identify song types (Sinnott et al., 1980;

Weary, 1990) and the robustness of song classification in noise (Wiley and Richards, 1982; Guilford and Dawkins, 1991). For example, if a subject consistently responds to Stimulus X with Answer Y, one could systematically manipulate the properties of the stimulus to determine the structural space that the subject recognizes as X. Logue's (2007b) chimeric song playback is the only study that we know of to take this approach.

DISCUSSION

Duetting behavior occurs at two levels of organization: the individual and the pair. Our thesis is that this distinction is foundational to developing a correct and complete understanding of duetting. It is also a reminder to think and write precisely. Vague terms, like the often-used "function of duetting," should be replaced by precise ones, like "the function of song answering in males." Students of collective behavior, the control of behavior, vocal interaction, and the evolution of social behavior have developed theory and research methods to address behavior across levels of organization. Duetting researchers can adopt and, where necessary, modify these ideas to apply them to vocal duetting systems. Not only will this approach advance duetting research, it will allow advances within duetting research to feed back into related fields. Such cross-fertilization has the potential to transform duetting research from a "niche market" into a more broadly relevant field of research.

The research framework and insights in this paper have applications outside of avian duetting research. Vocal chorusing involves all of the same behaviors as duetting (initiation, answering, phrase choice, and fine-scale timing), and so virtually all of the ideas that we discuss above may be applied to chorusing research. Duetting also has strong parallels with countersinging between neighboring territory holders. As Hall (2009) recognized, duetting and countersinging are both "interactive processes that involve time- and pattern-specific relationships among the exchanged signals" (p. 170; see also Todt and Naguib, 2000). Research on countersinging (e.g., song type matching, overlapping, communication networks) might benefit from many the ideas we have discussed. For example, agent-based models might be used to model communication networks, and the ontogeny of the rules that govern countersinging might be studied in both early life and as adult birds develop relationships with their neighbors.

The ideas discussed in this paper are also applicable to duetting and chorusing primates, such as gibbons (Geissmann, 2002), monkeys (Müller and Anzenberger, 2002), and lemurs (Baker-Médard et al., 2013). Of course, there is another group of primates that has evolved an extraordinarily complex form of interactive vocal communication. In spite of the obvious parallels, however, the study of conversation in humans has had minimal crossover with duetting research (Salwiczek and Wickler, 2004; Logue and Stivers, 2012; Ghazanfar and Takahashi, 2014). It is our hope that with increased attention to the levels of organization, research on vocal duetting will help us understand this most human form of collective behavior.

AUTHOR CONTRIBUTIONS

DL conceived of the paper, wrote the first draft of everything but Insight #3, and edited the entire MS. DBK wrote the first draft of Insight #3, and edited the entire MS.

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Female Song in New World Wood-Warblers (Parulidae)

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Recent advances have revealed that female birdsong is widespread and multifunctional. Female song was likely ancestral among songbirds and persists in many lineages today. Nevertheless, many species lack female song, and researchers are interested in understanding the selective factors that promote and counter the persistence of this trait. Female song is associated with life-history traits including year-round territoriality, non-migratory behavior, sexual monochromatism, and monogamy. Most studies examining these relationships have looked at clades with a migratory ancestor and have found that gains of migratory behavior are strongly correlated with losses of female song (and duetting). Here, we ask if the reverse pattern exists: in a large clade of songbirds with a migratory ancestor, do losses of migratory behavior correlate with gains of female song and visual signaling traits? We investigated correlations between female song, migration, and dichromatism in 107 species of New World Warblers (Family Parulidae). All of these species are predominantly monogamous and territorial when breeding, 50 (47%) are migratory, 49 (46%) are monochromatic, and 25 (23%) show female song. On a robust genetic phylogeny maximum likelihood methods recover migration and monochromatism as the ancestral state in warblers. Female song is generally not reconstructed as present in any deep nodes of the phylogeny, suggesting that most extant species with female song evolved this trait independently and relatively recently. Gains of female song do not correlate with losses of migration. Losses of dichromatism do correlate with losses of migration. Thus, in this clade, visual signals are associated with sedentary vs. migratory lifestyles, but female acoustic signals are not. Our results show a different pattern from that seen in similar studies and support the hypothesis that losses, but not gains, of female song are driven by life history.

Keywords: female song, dichromatism, migration, correlated evolution, Parulidae

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INTRODUCTION

For most of the history of its academic study, song in birds has been considered an almost exclusively male trait (Langmore, 1998; Catchpole and Slater, 2008). Much recent work has corrected this bias and found that while male song is certainly more common in some geographical regions, female song is widespread among songbirds (Odom et al., 2014) and can have important functions in many (if not all) of the same contexts as male song: defense of a territory, mate attraction, maintenance of the pair bond, and mate guarding (Hall, 2004; Slater and Mann, 2004).

Abbreviations: ML, maximum likelihood.

In addition to understanding the functions of female song, researchers are interested in the evolutionary history, and ecological correlates of female song (Price, 2009; Odom et al., 2014). Observers have long noted that female song is more common in tropical and sub-tropical areas than it is in temperate regions (Slater and Mann, 2004). Traditionally this pattern is attributed to the fact that resources in tropical regions are divided among more individuals, making them available at relatively low levels year-round and are therefore defended by both members of a territorial pair, leading to a general convergence of sex roles (Morton, 1996).

A recent surge of interest in this topic has supported the idea that female song is associated with life-history traits that are common in tropical areas, including year-round territoriality and/or non-migratory behavior, sexual monochromatism, carotenoid dichromatism, and monogamy (Malacarne et al., 1991; Garamszegi et al., 2007; Benedict, 2008; Price, 2009; Price et al., 2009; Logue and Hall, 2014). In particular, gain of migratory behavior is strongly correlated with loss of female song (including duetting; Price et al., 2009; Logue and Hall, 2014). "Migration" may be best thought of not as one trait, but as an attribute that represents an amalgam of traits (navigational abilities, flight behavior, etc.) that are likely to affect general life history strategies (Zink, 2002). Migratory species tend to be dichromatic (Hamilton, 1961), have higher divorce rates (Jeschke and Kokko, 2008), and have higher rates of extra-pair paternity (Spottiswoode and Møller, 2004) than sedentary species; all of these life history characters might be summed up as factors contributing to divergence in sex roles, and potentially as factors selecting against the presence of female song in migratory groups.

The New World blackbirds (family Icteridae) are the best-studied group in regards to the life history correlates of female song (Hofmann et al., 2008; Price, 2009; Price et al., 2009). Barring the basal meadowlark clade, the ancestor of the rest of the New World blackbirds is inferred to have been a sedentary, monochromatic species with singing females. Many blackbird species retained this sedentary, tropical lifestyle along with monochromatism, and female song. Species that lost female song generally did so in conjunction with gains in migration, losses of bright female plumage, and/or changes in breeding strategy, such as switching from monogamy to polygyny or colonial breeding (Price, 2009). Thus, studies of this group have revealed much about when and why female song is lost in species descended from a sedentary common ancestor with female song. A comprehensive study of male-female duet presence in songbirds also found that losses of female song were correlated with gains of migration, and indicated that losses of female song are much more common than are gains of this trait (Logue and Hall, 2014). This pattern fits well with the result that female song is ancestral in all songbirds (Odom et al., 2014).

While existing evidence suggests that the lack of female song in temperate regions likely represents losses of this trait, it is less clear if gains of female song are common and if they are driven by the same selective forces as losses of female song. Here, we investigate this question using a large clade (107 species) of new world songbirds whose ancestor is inferred to be migratory (Winger et al., 2014). The New World wood-warblers (family Parulidae) are the sister group to the New World blackbirds (Barker et al., 2015). Like blackbirds, warblers are represented by a mix of non-migratory and migratory species as well as monochromatic and dichromatic species, and include members with females reported to sing regularly or rarely. Unlike among blackbirds, the stronghold of warbler diversity is in North America and the base of the warbler phylogeny is dominated by migratory species. Additionally, apart from whether they migrate, nearly all warblers have similar life histories: they are socially monogamous, defend territories during the breeding season, and build cup nests on or near the ground.

Making use of recently published molecular phylogenies resolving the relationships among warblers (Lovette et al., 2010; Barker et al., 2015) we reconstructed the evolution of female song in this group and we tested for correlations among female song, dichromatism, and migratory behavior to investigate the possible relationships among these life history traits. Following the hypothesis that non-migratory, monogamous species with similar sex roles are more likely to express female song (Morton, 1996; Benedict, 2008; Price, 2009), we predicted that gains of female song in warblers would be strongly correlated with losses of migration and losses of dichromatic plumage.

METHODS

Literature Search

We searched the literature for any reference to female song in each of the 107 warbler species represented in Lovette et al.'s (2010) molecular phylogeny. We used primary literature, field guides, Neotropical Birds Online, the Birds of North America Online, and the Handbook of the Birds of the World (Del Hoyo et al., 2011). Using Web of Science, we searched for the common name, old scientific name, and recently revised scientific name of each species (per Lovette et al., 2010). Given the difficulty of detecting female song and an overrepresentation of North American species in the literature, we attempted to take into account the research effort associated with each species. Accordingly, we scored a species as being "well-researched" if we could find five or more publications where the focal species was being directly observed by a researcher and "poorly researched" if we could not. We used this method because it reflects the bimodal nature of our search results. Searches generally either returned more than five papers focused on a single species, or simply returned publications where a species is mentioned only as part of a list and was never the subject of direct focal observation. Occasionally searches returned one or two brief reports focused on a single species, but these tended not to be focused on song behavior. We searched English-language sources only.

We scored a species as having female song if we found any reference to a singing female, including solitary reports (Appendix 1 in Supplementary Material). Not all species are reported to sing regularly, so we marked a species as being a "rare" singer if accounts described female song as rare or uncommon or if main sources (like the Birds of North America reports) do not mention female song but a report exists for that species. We scored a species as not having female song if species accounts specifically state that females do not sing, or if we scored that species as being well-researched and we found no mention of female song. We scored female song as "unknown" for species in which we could find no report of female song but were also poorly researched.

We looked at photographs and plates of each species to score carotenoid and melanin dichromatism (Appendix 1 Supplementary Material). Melanin and carotenoid pigments are acquired through different modes and thus are thought to carry different signals in birds (Badyaev and Hill, 2000). We counted plumage colors that might be described as black, brown, gray, rufous, cinnamon, or buff as melanin coloration and colors that might be described as reds, oranges, or yellows as carotenoid coloration. Plumage dichromatism was scored on a scale of 0–2 for both melanin and carotenoid plumage characters, with 0 indicating no difference between males and females, 1 indicating a weak difference, and 2 indicating a striking difference between males and females. For example, we scored *Geothlypis trichas* as carotenoid monochromatic (0) since females and males appear equally brightly yellow but are strikingly melanin dichromatic (2), with females lacking the black mask characteristic of males. We scored only the nominate subspecies for each species. Two raters scored plumage characters for all species. A third rater scored species where there was disagreement, and a consensus score was assigned to each species. This approach is supported by research showing that painted plates and photographs are reliable sources of biologically relevant patterns in birds (Møller and Birkhead, 1994). Like most species of birds, parulid warblers have plumage that reflects UV light, which can result in humans estimating a bird to be monochromatic when it is actually dichromatic (i.e., one sex has a UV patch). However, a study comparing human estimates of dichromatism with spectrophotometer estimates of dichromatism found that, in warblers, human estimates are reliable, indicating both males and females have similar UV reflectance or that species dichromatic in the UV spectrum are also dichromatic in the visible light spectrum (Armenta et al., 2008).

We used the sources described in our literature search methods above to determine whether a species is migratory or sedentary (Appendix 1 in Supplementary Material). We found one species to be described as an altitudinal migrant, the arrowhead warbler, *Setophaga pharetra*, and two species to be described as partial migrants (not all individuals migrate), the pine warbler, *Setophaga pinus*, and the painted whitestart, *Myioborus pictus*. We counted altitudinal or partial migrants as being "migratory" since they are still making seasonal movements which require many (if not all) of the same tools used by a long-distance migrant (i.e., a compass, restlessness, etc.)

Phylogenetic Analyses

We determined the ancestral states of female song, migration, and plumage dimorphism in Parulidae using maximum likelihood (ML; one or two parameter Markov k-state) using the stored models in the ancestral state reconstruction package of Mesquite 3.02 (Maddison and Maddison, 2015) with a recent molecular phylogeny of the wood-warblers (Lovette et al., 2010). A one parameter likelihood model assumes the backward and forward rates of evolution are the same (symmetrical). That is, the rate of gain of a trait is the same as the rate of loss. A two parameter likelihood model has a backward rate different from the forward rate (asymmetrical). A likelihood ratio test was used to determine which likelihood model (symmetrical or asymmetrical) to use. This test compares the one and two parameter reconstructions of the same character on the same tree to determine whether the complex two parameter model fits the data significantly better than the simpler one parameter model. We used an alpha of 0.05 as our cutoff for "significantly better fit." It should be noted that asymmetrical models can only have two character states, so we did not use an asymmetrical model for the three-state characters carotenoid 2 and melanin 2. We called a particular character state to be likely at a given node if it was reconstructed as being at least 70% likely, i.e., had a proportional likelihood of 0.70 or greater. The proportional likelihood is the probability that a given node was a particular state, calculated by adding the two likelihoods and taking the ratio of one to the total. For maximum likelihood models in general, models with a value closer to 0 are better than models farther away from zero (e.g., a model with log likelihood −20 is better than a model with log likelihood −30). We discuss increases in the prevalence of a state as "gains" and decreases as "losses," although we did not apply a statistical test to our character state reconstructions to do so as Mesquite does not currently support statistical analysis of character state reconstructions.

Coding scheme can be extremely important when reconstructing character histories (e.g., Thorpe, 1984; Wilkinson, 1995; Strong and Lipscomb, 1999). We therefore used multiple coding schemes to reconstruct character histories in Mesquite in order to evaluate the possible effect of coding strategy on the reconstruction of female song. Coding schemes are listed in **Table 1**.

Female song coding scheme 1 included three possible character states (song present, song absent, and unknown), while all of the other female song coding schemes included only two possible character states (song present, song absent). All coding schemes were used in ancestral state reconstruction, and coding schemes with only two possible character states were used in analyses of trait correlations. We included female song coding schemes 2, 3, and 4 in order to assess the potential effects of low research effort on some species. We included female song coding scheme 5 because rare female song may have little biological importance. Melanin and carotenoid dichromatism were coded in two ways: a scheme with all three states for use in ancestral state reconstructions and a scheme with two states for use in correlation analyses. Melanin dichromatism was split into none/weak (0/1) and striking (2) since most species had striking dichromatism, while carotenoid dichromatism was split into none (0) and weak/striking (1/2) since only two species had striking carotenoid dichromatism. We then pruned the tree to exclude species with low research effort and compared the pruned reconstruction with the full dataset. In general, the pruned tree was simpler to interpret with fewer equivocal nodes, but the overall pattern of gains and losses were the same, and so we do not report ancestral state reconstructions from pruned trees.

TABLE 1 | Trait coding schemes and explanations.

Scheme name	Coding scheme	Explanation
Female song 1	0: absent, 1: present, ?: unknown	Incorporates missing information, coded for ancestral state reconstructions
Female song 2	0: absent, 1: present, ?: absent	All missing data interpreted as "absent"
Female song 3	0: absent, 1: present, ?: present	All missing data interpreted as "present"
Female song 4	0: absent 1: present ?: 0 or 1	Randomly assign missing data as present or absent
Female song 5	0: absent, 1: present, ?: absent	Missing data and rare reports interpreted as absent; song counted as present only if females regularly sing
Migration	0: sedentary, 1: migratory	Any seasonal movement counted as "migratory"
Overall dichromatism	0: dichromatic, 1: monochromatic	Any plumage difference between males and females counted as dichromatic
Carotenoid dichromatism 1	0: no dichromatism, 1: weak or striking dichromatism	Any dichromatism, coded for correlation analysis
Carotenoid dichromatism 2	0: none, 1: weak, 2: striking	Levels of dichromatism coded for ancestral state reconstructions
Melanin dichromatism 1	0: none or weak, 1: striking	Weak vs strong dichromatism, coded for correlation analysis
Melanin dichromatism 2	0: none, 1: weak, 2: striking	Levels of dichromatism coded for ancestral state reconstructions
Research Effort	0: low, 1: high	Species with fewer than five observational references were considered poorly researched

Mesquite interprets a "?" as indicating missing data.

We used Pagel's method (1994) to test for correlated evolution between two characters in the correlation package of Mesquite 3.02. Pagel's method takes phylogeny into account as it looks for correlated changes in character states by testing between an independent and dependent model of character evolution on a tree. Two characters are correlated (or not independent from each other) when the dependent model (assumes traits are changing together with eight parameters) is significantly different from the independent model (assumes traits are not changing together with four parameters). The *p*-values are generated from parametric bootstrapping of the two models using a likelihood ratio test over many simulations (requiring a minimum of ∼1000 simulations). This method requires all characters to be binary and to have no missing data, so we did not run this analysis on female song 1, carotenoid dichromatism 2, or melanin dichromatism 2. Random assignment of female song to species with missing data (song coding scheme 4) and assigning missing data as indicating presence of song (song coding scheme3) yielded very poorly supported trees (log likelihood: −71.67 and −70.77, respectively; all other trees we used had log likelihoods >−58) so these coding schemes were not used in subsequent analyses. We tested for correlated evolution between female song 2, 5, migration, any plumage dimorphism, carotenoid dimorphism, and melanin dimorphism, as well as between migration and overall plumage dimorphism, migration, and carotenoid dimorphism, and migration and melanin dimorphism (3000 simulations, 1000 searches, any effect). To assess the potential effects of missing data we pruned the tree to remove all species with "unknown" female song. We conducted two preliminary correlation analyses between female song 2, 5, and migration using the pruned tree, but these results were not appreciably different from the results using the full tree, so we used the full tree for all subsequent correlations. The patterns of evolution of female song reconstructed using the full tree for female song coding schemes 2 and 5 were not different from reconstructions obtained from the pruned tree.

RESULTS

Literature Search

Of the 107 species included in Lovette et al.'s (2010) phylogeny of the Parulidae 50 (46.7%) species are migratory, 49 (45.7%) species are monochromatic, and 25 (23.3%) species have at least one report of female song. Of these 25 species, 12 are reported to sing regularly, and seven of those 12 species are duetters. Of the seven duetting species, six species are sedentary and monomorphic (only *M. pictus* is not, see the Appendix in Supplementary Material for the full data set).

We marked 41 (38.3%) species as "unknown" for female song due to poor research effort. Only 19.5% (8) of the poorly researched species were migratory (including two extinct species, *Vermivora bachmanii* and *Leucopeza semperi*), while 51.2% (21) of them were monochromatic. Among the 73 relatively well-researched species in the dataset, 34.2% had female song, 36.8% (28) were monochromatic and 57.5% (42) were migratory.

Of the species scored as dichromatic, 17 species were weakly carotenoid dichromatic, two species were strikingly carotenoid dichromatic, 22 species were weakly melanin dichromatic, and 34 species were strongly melanin dichromatic. Two species were weakly dichromatic because the females had carotenoids where the male had none (*Setophaga cerulea* and *Setophaga caerulescens*).

Phylogenetic Analysis

Ancestral State Reconstructions

Character histories for female song 1, 2, 4, and 5 were reconstructed using the two-parameter model. Female song is generally not reconstructed as present in any deep nodes of the phylogeny, indicating that most extant species with female song evolved this trait independently and relatively recently (**Figures 1**, **2**).

If we code female song as unknown in species where there are no explicit reports on the presence or absence of female song (female song 1), we recover many estimated gains of female song and few losses (two-parameter log likelihood: −45.95). The one clade where female song appears to be relatively ancestral is within the genus *Myiothlypis*. The deepest node (representing the ancestral warbler) has a proportional likelihood of 0.32 that it had female song.

Female song 2 (all missing data coded as "female song absent") yields an almost identical reconstruction to female song 1, albeit not as strongly supported, with *Myiothlypis* again containing a relatively ancestral node that may have had female song (two parameter log likelihood: −57.62, proportional likelihood: 0.72). The deepest node has a proportional likelihood of 0.27 that it had female song. Pruning the tree to remove species with unknown song status due to low research effort results in the same overall reconstruction, with all taxa (including *Myiothlypis*) being largely equivocal for female song (**Table 2**).

Using female song coding scheme 5 (only common female song) produced a tree with only 12 species with female song, nearly all of which appear homoplasious, and with no deep ancestral female singers (two parameter log likelihood: −38.13). Removing species with low research effort results in a marginally better reconstruction of female song (log likelihood: −36.2; **Table 2**).

Overall, the likelihood that ancestral nodes had female song increases when we indicate missing data, but the pattern of evolution of female song is fairly resistant to changes in coding scheme using likelihood methods and under nearly every scheme results indicate many gains of song, but a *rate* of loss that is at least twice as large as the *rate* of gain (e.g., female song 2 forward rate: 12.9, reverse rate: 42.3). Removing species with missing data does not change the overall pattern, although it does generally improve the likelihood values of the reconstructions (**Table 2**).

Migration is recovered as the ancestral state in warblers (**Figures 2**,**3**). Maximum likelihood strongly supports a migratory ancestor (log likelihood: −36.79, proportional likelihood migratory: 0.98). Monochromatism is also recovered as the ancestral state in warblers (**Figures 1**, **3**, **4**; log likelihood: −23.84, proportional likelihood monochromatic: 0.98). Carotenoid dichromatism (weak and strong) appears to have been gained many times with strong carotenoid dichromatism evolving only twice (in *Setophaga ruticilla* and *Setophaga fusca*). Melanin dichromatism, on the other hand, is predominately represented by strong differences between males and females with 34 species exhibiting striking dichromatism and 22 species exhibiting weak dichromatism (**Figure 4**). More dichromatic species were melanin dichromatic than carotenoid dichromatic.

Correlation Analyses

Most correlation analyses were conducted using song coding scheme 2 (female song coded as either present or absent) since this coding scheme includes all reports of female song and using other coding schemes doesn't change the resulting correlations. Using the full parulid tree (including species with missing data coded as song absent) song coding scheme 2 was not correlated with migration (3000 simulations, $p = 0.16$, **Figure 2**), general dichromatism (3000 simulations, $p = 0.09$, **Figure 1**), melanin dichromatism 1 (1000 simulations, $p = 0.77$, **Figure 4**), or carotenoid dichromatism 1 (1000 simulations, $p = 0.98$, **Figure 4**). Removing species with low research effort (i.e., using a pruned tree) did not change whether or not a correlation with female song 2 was significant (migration— $p = 0.11$, monochromatism— $p = 0.19$; **Table 3**). Since removing species with low research effort did not change character correlation results, we used the full tree in all subsequent analyses. Female song 5 (rare reports coded as "absent") was not correlated with migration (1000 simulations, $p = 0.38$) or plumage dichromatism (1000 simulations, $p = 0.34$; **Table 3**). As our female song 3 and 4 coding schemes are biologically unrealistic and the character reconstructions were poorly supported we did not run correlation analyses for them.

Migration and dichromatism were significantly correlated with each other (3000 simulations, $p < 0.001$; **Figure 3**, **Table 3**). More specifically, migration was significantly correlated with both melanin dichromatism 1 (1000 simulations, $p = 0.001$) and carotenoid dichromatism 1 (1000 simulations, $p = 0.003$; **Table 3**).

Transition Rates

The transition rates of the four possible character state combinations in each of three correlations (female song 2 vs. migration, female song 2 vs. any plumage dichromatism, and migration vs. any plumage dichromatism) were compared to assess the relative rates of transition to and from particular states within a given correlation (**Figure 5**).

When comparing female song and migration, the largest transition was overwhelmingly from being migratory and having song to losing song (q42 = 79.54, **Figure 5A**); this transition occurred about 6.5 times more frequently than the opposite transition (gaining song while migratory). The largest relative rate among two states was switching from being sedentary and having female song to losing female song, with losses occurring 21 times more frequently than gains (**Figure 5A**).

Similarly, song was lost much more frequently than it was gained when a species is dichromatic (rate of loss ∼16.5 times larger than gains) and to a lesser extent when a species is monochromatic (rate of loss ∼4 times larger, **Figure 5B**). Overall transition rates between presence and absence of female song in monochromatic species were larger than in dichromatic species (q13 = 66.75, **Figure 5B**). Plumage characters seem resistant to change relative to song characters and had low transition rates overall, with the highest being the transition from dichromatic to monochromatic without female song (q21 = 10.43, **Figure 5B**). Female song was not significantly correlated with migration or plumage dichromatism, so the results of the transition rates between these states should be interpreted with caution.

The largest transition rate of the correlation between migration and any plumage dichromatism was the transition from sedentary to migratory in dimorphic species (q24 = 31.39,

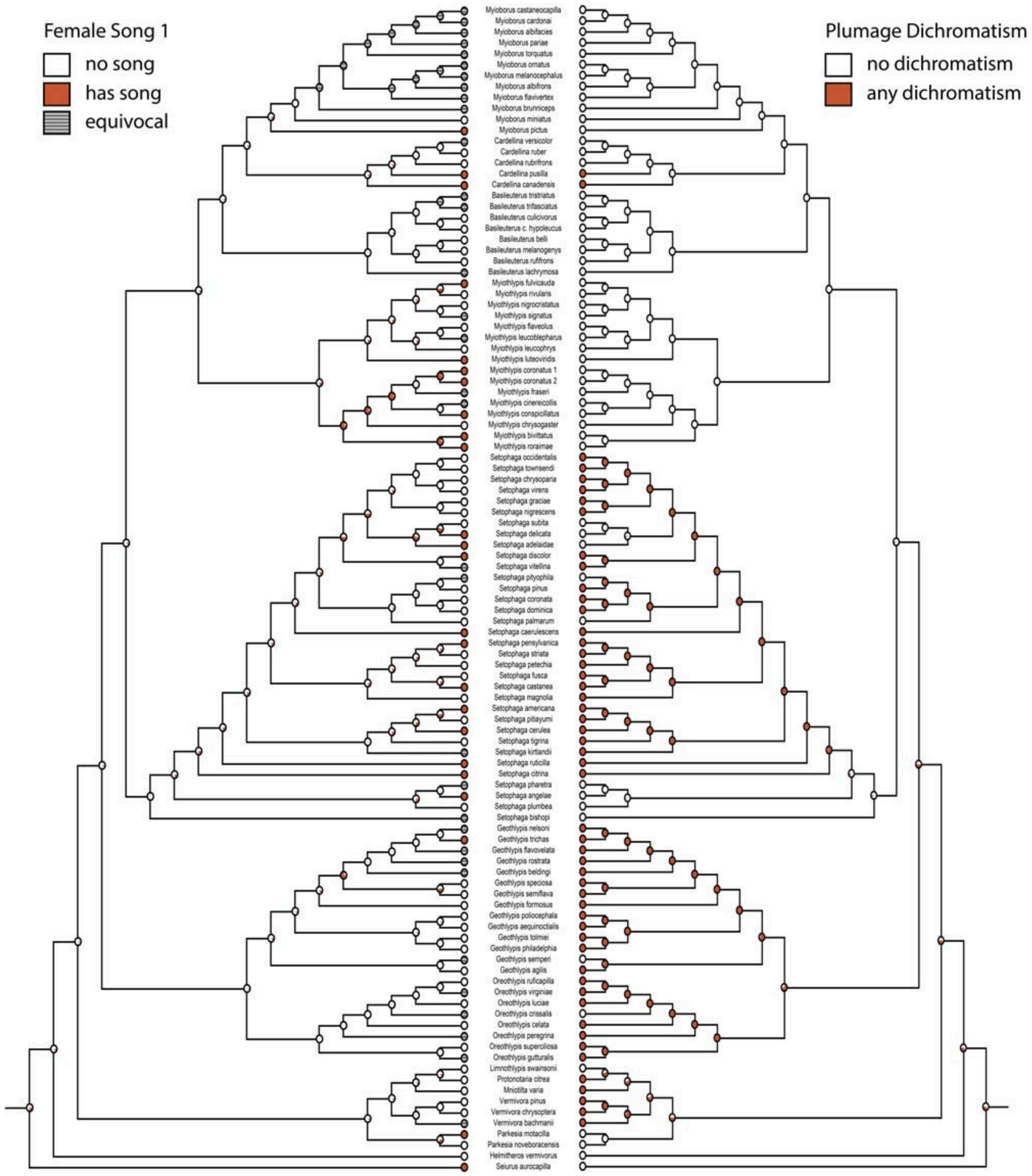

FIGURE 1 | Ancestral state reconstruction with female song 1 (mk2, log likelihood: −45.95) on the left mirrored with any plumage dichromatism (mk1, log likelihood: −56.91) on the right. Red circles have female song or are dichromatic and white circles do not, gray circles are equivocal. Pie charts indicate proportional likelihoods.

Figure 5C), occurring about twice as frequently as the reverse transition (q42 = 14.30, **Figure 5C**). The next largest transition was from dichromatic to monochromatic in sedentary species (q21 = 20.70, **Figure 5C**); transitions in the opposite direction (gaining dichromatism while sedentary) almost never happens (q12 = 0.001, **Figure 5C**).

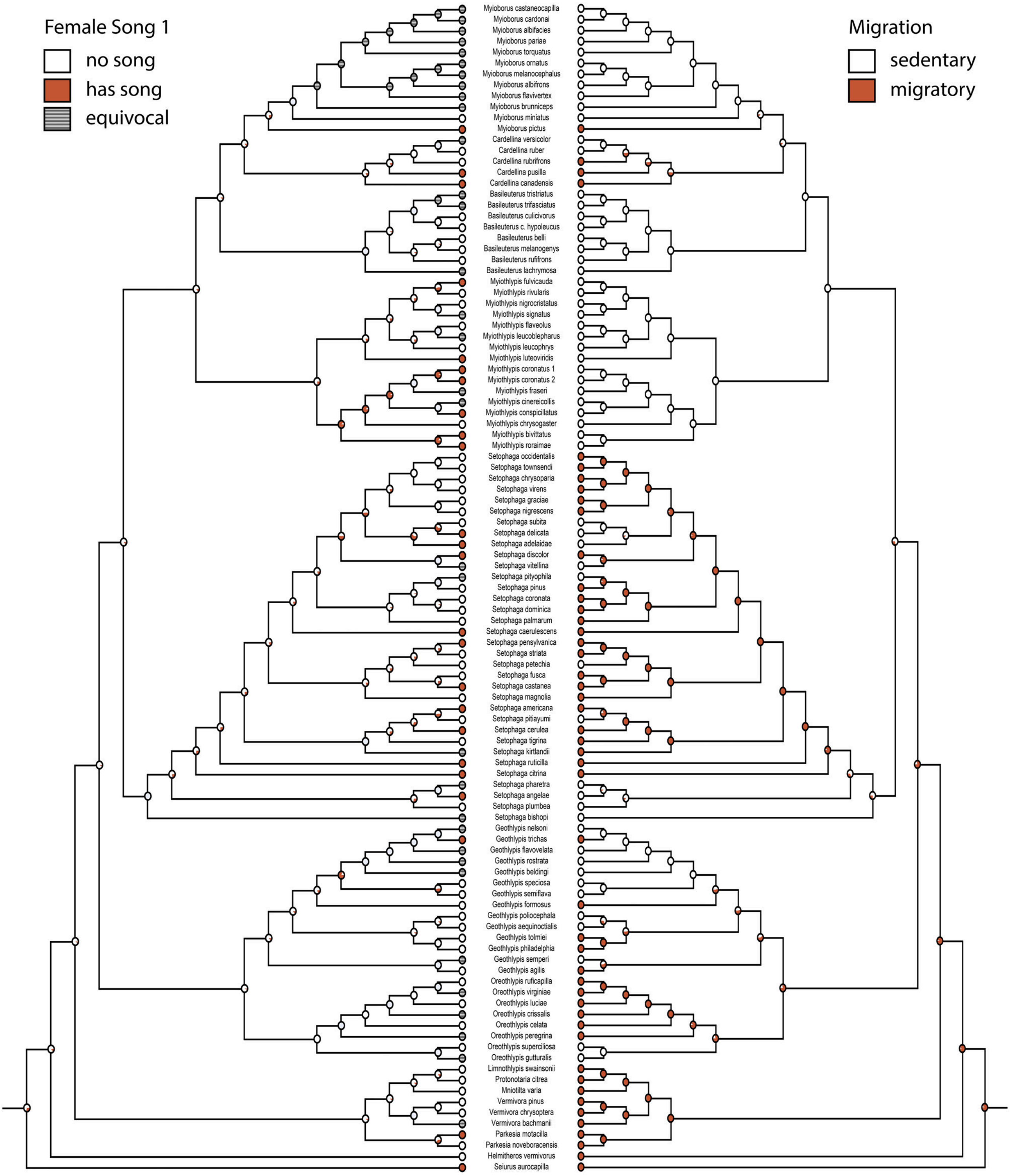

FIGURE 2 | Ancestral state reconstruction for female song 1 (mk2, log likelihood: −45.95) mirrored with migration (mk1, log likelihood: −46.81). Red circles have female song or are migratory, white circles do not. Gray circles are equivocal. Pie charts indicate proportional likelihoods.

TABLE 2 | Character state reconstructions.

Character state	ML full	ML pruned
Female song 1	−45.95*	−46.05*
Female song 2	−57.62*	NA
Female song 3	−70.67	NA
Female song 4	−71.67*	−46.66*
Female song 5	−38.13	−33.52
Migration	−56.91	−41.22
Any dichromatism	−46.81	−33.45
Carotenoid dichromatism 1	−49.27*	−33.46*
Carotenoid dichromatism 2	−68.94	−48.8
Melanin dichromatism 1	−43.93*	−38.37*
Melanin dichromatism 2	−86.99	−67.79

ML-values shown are based on whether the two-parameter model was significantly different from the one-parameter model (indicated with an asterisk). ML, maximum likelihood.

DISCUSSION

Distribution of Female Song in Parulidae

Female song is uncommon but not rare in Parulidae with 23% of species having at least one report of a singing female. Unexpectedly, given current hypotheses and previously published literature on the distribution of female song in birds (Morton, 1996; Garamszegi et al., 2007), these species are distributed relatively evenly among migratory and sedentary clades and show no associations with sexual monochromatism vs. dichromatism.

It can be argued that female song is rare, aberrant, or biologically unimportant in species for which we found only one or a few reports of female song. Most Parulid species with frequent female song (12 species, 11% of Parulids) are in the large clade consisting of *Myiothlypis*, *Basileuterus*, *Cardellina*, and *Myioborus* warblers. This group is generally monochromatic and sedentary and thus conforms to current hypotheses on life history correlates associated with female song. Indeed, all of the duetting species occur in this large clade and duetters account for seven of 12 species reported to sing regularly. However, not all species with frequent female song conform to this pattern, and removing species with rare reports does not change character state reconstructions, only somewhat improves the likelihood of the reconstructions, and does not result in significant correlations between female song and either migration or plumage dichromatism.

Ancestral State Reconstructions

Overall the ancestral warbler is reconstructed to not have had singing females. This holds true using different coding schemes for the presence or absence of female song to account for differential research effort. Therefore, we think it much more likely that female song has been repeatedly gained in warblers. Even if we restrict our analysis to species well known to sing or duet, there is not just one origin of this behavior but rather multiple independent origins.

There are only two species branching early in parulid evolution that have reports of female song, the ovenbird (*Seiurus aurocapilla*) and the Louisiana waterthrush (*Parkesia motacilla*). Female song in both of these species is thought to be rare, and is likely aberrant in the ovenbird, an extremely well-studied species with only one report of a singing female (Hiatt, 1943). When rare reports of female song such as these are considered aberrant behavior, then the ancestor of all New World warblers does not reconstruct with female song. Therefore, we conclude that the ancestral warbler did not have frequent female song and we think it is very likely that it also did not have infrequent female song.

Prior studies reconstructing the ancestral state of female song in New World blackbirds (Icteridae) found song to be equivocal (Price, 2009). This seemed to be driven by the most basal ingroup Icterid clade, the meadowlarks and allies, which do not have female song. Given that Parulidae is the sister taxon to Icteridae, it now seems likely that the common ancestor of these groups did not have female song. However, it is also possible that the ancestor of all Icteridae (and possibly Parulidae) had female song and the loss of female song is derived in the meadowlark clade and also occurred relatively early in warbler evolution.

Migration and Plumage Dimorphism

The only characters strongly correlated with each other in our analyses were migration and plumage dimorphism. This is in accordance with other studies of life history correlates of migration in parulids (Cardoso and Hu, 2011; Simpson et al., 2015) and other birds (Hamilton, 1961; Hofmann et al., 2008; Price, 2009; Price et al., 2009). There are two major (and not necessarily mutually exclusive) hypotheses attempting to explain widespread dichromatism in migratory species. Environmental factors, especially predation, may select for decreased plumage elaboration in migratory females since they are generally not defending territories on the breeding grounds (Badyaev and Hill, 2000). Alternatively, migratory species have less time to pair and breed, so changes in female plumage may be selected for reduced male aggression toward the female and to facilitate rapid pair-bonding (Hamilton, 1961). Generally, female dichromatic warblers are less colorful than males, but are usually not cryptically colored and may overall look very similar to males. Given this pattern, it may be possible that there is selection for reduced male aggression, but not for the cryptic plumage seen in other groups, such as blackbirds, where dichromatism is driven by losses of elaborate carotenoid plumage in females (Hofmann et al., 2008).

Migration and Female Song

In our analyses the ancestral warbler is reconstructed to be a migrant. This is in agreement with a recent study of the Emberizoidea, which reconstructed the ancestral state of all warblers as north temperate breeders that migrate to the Neotropics (Winger et al., 2014). Migration is lost in the more recently derived Neotropical clades (*Myiothlypis, Basileuterus, Cardellina,* and *Myioborus*) and only regained twice (in the ancestor of the *Cardellina* group and in *M. pictus*). Incidentally, this finding is contrary to the long-held hypothesis that

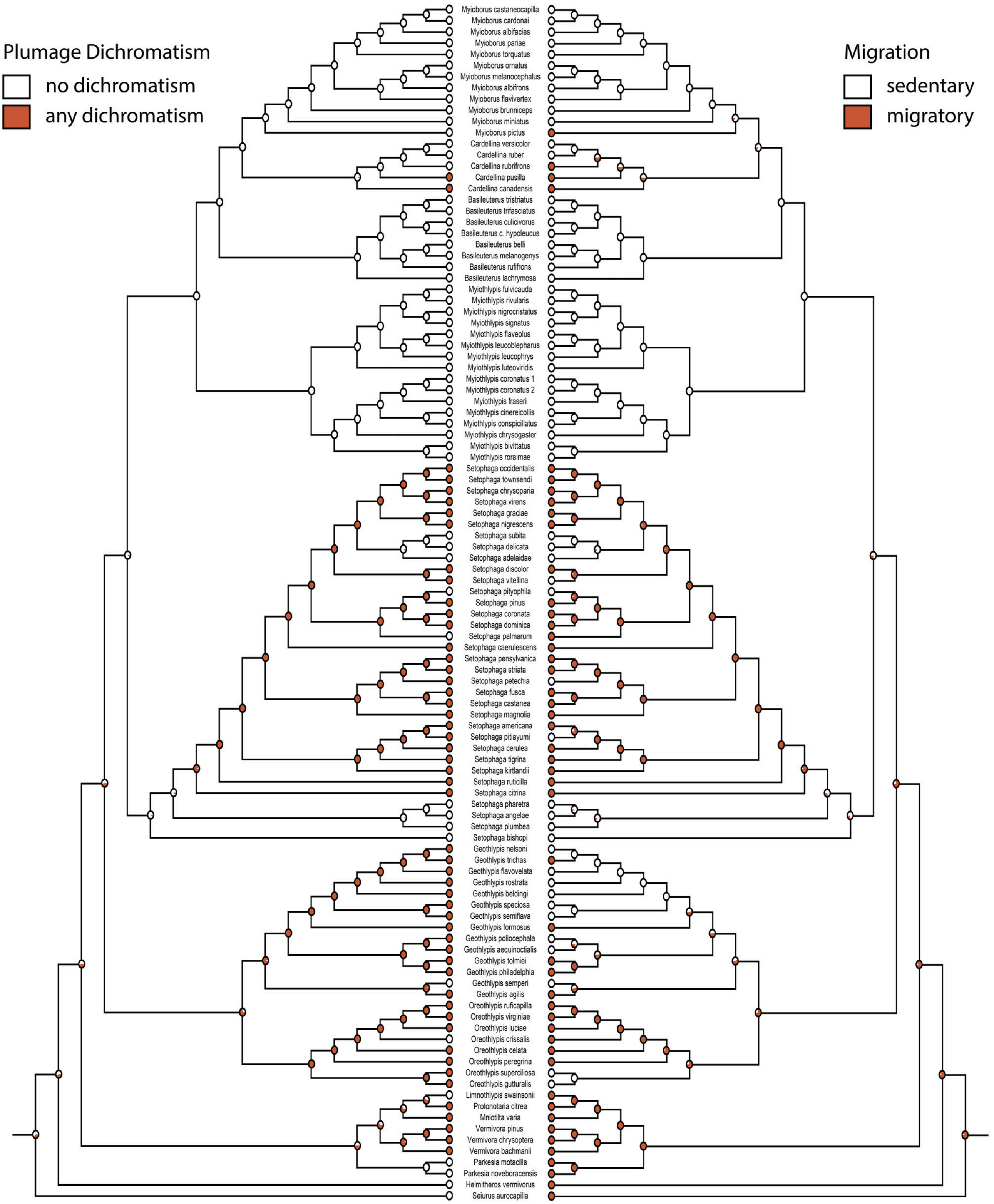

FIGURE 3 | Any plumage dichromatism (mk1, log likelihood: −56.91) on the left mirrored with migration (mk1, log likelihood: −46.81) on the right. Red circles are dichromatic or migratory, white circles are not. Pie charts indicate proportional likelihoods.

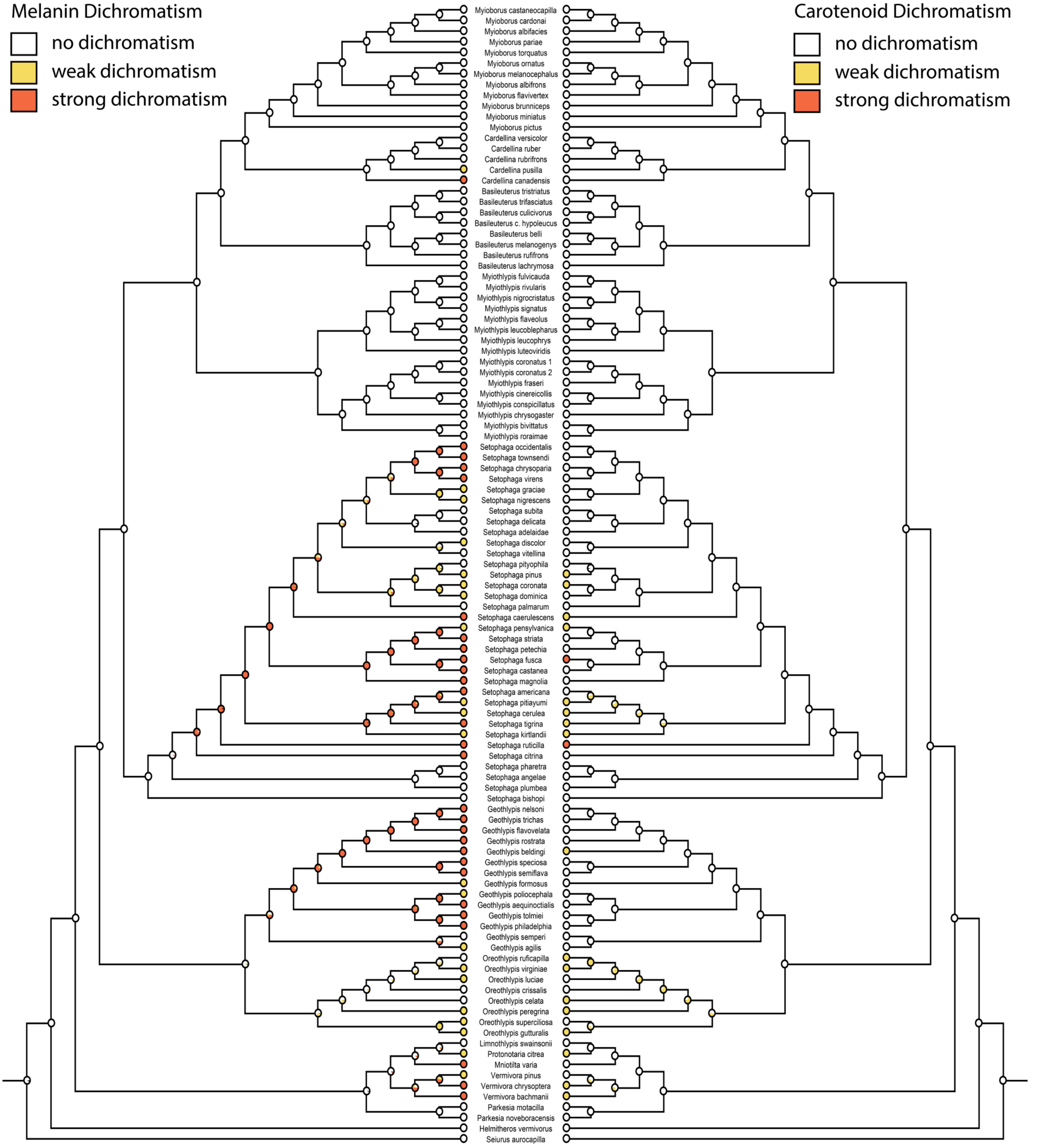

FIGURE 4 | Ancestral state reconstructions of melanin dichromatism 2 (mk1, log likelihood: −86.99) on the left mirrored with carotenoid dichromatism 2 (mk1, log likelihood: −68.94) on the right. White circles are monochromatic, yellow circles weakly dichromatic, and red circles strongly dichromatic.

migration in birds evolves in the tropics as an escape from competition pressure (Levey and Stiles, 1992; Rappole, 1995; Rappole and Jones, 2003), particularly given the likely dispersal of the ancestor of the Emberizoidea (including warblers) into north America from Eurasia over Beringia (Barker et al., 2004).

When counting all 25 parulid species with reports of singing females as indeed having common female song, our analysis

TABLE 3 | Results of correlation analyses using Pagel's correlation method.

Character 1	Character 2	Diff in log likelihood	*p*-Value	Tree type
Female song 2	Melanin dichromatism 1	0.08	0.77	Full
Female song 2	Carotenoid dichromatism 1	−1.72	0.98	Full
Female song 2	Any dichromatism	1.7	0.09	Full
Female song 2	Any dichromatism	0.76	0.19	Pruned
Female song 2	Migration	0.12	0.16	Full
Female song 2	Migration	0.95	0.11	Pruned
Female song 5	Any dichromatism	0.09	0.344	Full
Female song 5	Migration	1.29	0.388	Full
Migration	Any dichromatism	12.25	<0.001	Full
Migration	Carotenoid dichromatism 1	7.1	0.003	Full
Migration	Melanin dichromatism 1	9.98	0.001	Full

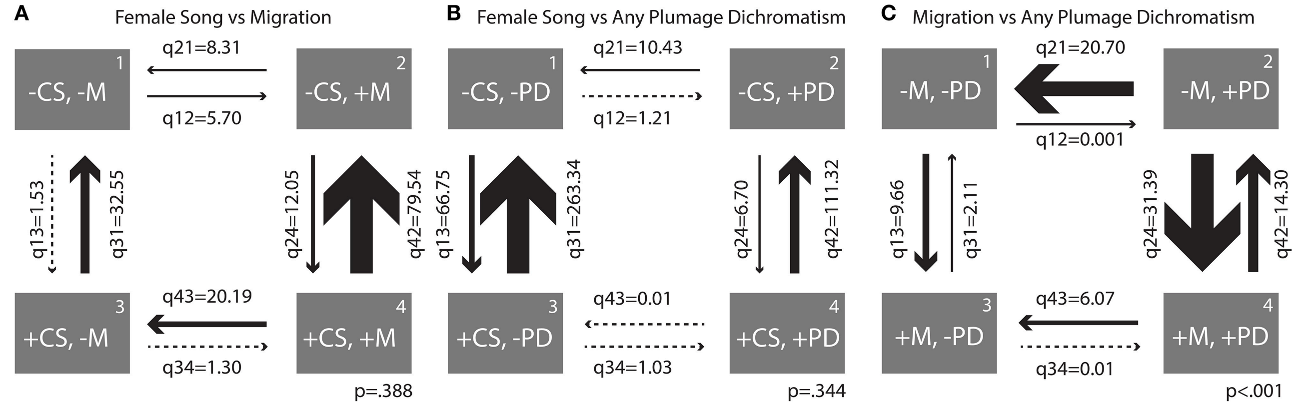

FIGURE 5 | Transition rates derived from correlation analyses between (A) female song 2 and migration, (B) female song 2 and any plumage dichromatism, and (C) migration and any plumage dichromatism. Arrows are scaled to the largest transition rate in each figure.

found that the evolution of female song is not correlated with losses or gains of migration in this group. Given that the ancestral warbler was likely migratory and had no female song, it's possible that female song is simply difficult to re-gain even if female song confers higher fitness in sedentary species. In a study comparing the evolution of duetting and migration, an ancestor that is both a duetter and a migrant was found to be five times more likely to lose duetting than to lose migration (Logue and Hall, 2014). In our study both migrants and non-migrants lose female song at rates 6–20 times higher than they gain it. Indeed, in all of our correlation analyses with female song, losses of this trait are more common than gains, regardless of migratory status or whether species are dichromatic. These patterns, coupled with the trait reconstructions suggest that female song is often gained independently, but is unstable and easily lost. Such losses are not correlated with migratory status or plumage dichromatism.

Sedentary species without female song may be in a fitness "valley" or on a relatively low fitness "peak" (Wright, 1982) that is difficult to get out of. Song was lost early in the evolution of warblers (possibly before they split from the blackbirds), so female song in warblers likely requires the reactivation of genes and physiological processes involved in song production. Females of many species can be induced to sing with the administration of testosterone (Kern and King, 1972; Nottebohm, 1980; Langmore, 1998) indicating the physiological machinery for song is often in place, but early developmental changes associated with testosterone production may be complicated and difficult to turn back on once lost.

For the purposes of understanding the evolution of female song, a characterization of territoriality in this clade might be informative. Species that are territorial year-round or that maintain a pair bond through the winter are exactly the sort of species we would predict to have female song (Benedict, 2008; Price, 2009). While most Neotropical migrants do not seem to be territorial in the winter, some, such as *Leiothlypis peregrina* (Birds of North America), hold winter territories, and are thus territorial year-round. Unfortunately, few data exist on these traits for most species of warblers, precluding more detailed analysis.

Plumage Dimorphism and Female Song

The ancestral warbler is reconstructed as being monochromatic in both separate analyses of carotenoid and melanin dichromatism, and is equivocal when reconstructing any dichromatism. We think it more likely that the ancestor was monochromatic given that extant species near the base of the warbler tree are dichromatic. Existing studies have shown that either monochromatism (Malacarne et al., 1991; Price, 2009) or dichromatism (Garamszegi et al., 2007) may be correlated with female song in birds. Our reconstruction indicates that parulid warblers underwent an early shift to dichromatism, but that gains of female song were not correlated with either losses or gains of melanin, carotenoid, or general dichromatism (**Table 3**). Female song appears much more labile than plumage characteristics as both transitions toward and away from female song were much larger than changes in plumage state (**Figure 5B**). Combined with other research, our study adds to the argument that there is no clear pattern of association between the presence of female song and dichromatism, suggesting that these traits may evolve relatively independently (Mason et al., 2014).

Our correlation analyses and character state reconstructions did not distinguish between monochromatic "dull" and monochromatic "ornamented" warblers. A recent study of the evolution of dichromatism in parulid warblers concluded that the common ancestor was monochromatic and bright (Simpson et al., 2015). Overall, our data suggest that dichromatism in this group is driven more by changes in melanin pigmentation than carotenoid pigmentation (often yellow) in females. Only two species were strongly dimorphic in carotenoid pigmentation, while 34 species were strongly dimorphic in melanin pigmentation. Whether the ancestor of warblers was ornamented or not is equivocal and should be the subject of future study.

In contrast with this pattern in parulids, plumage dichromatism in orioles (genus *Icterus*) is driven specifically by losses of bright plumage (both melanins and carotenoids) in females (Hofmann et al., 2008; Friedman et al., 2009). However, all male orioles are bright and/or saturated, so the ancestral state for male orioles is bright and relatively unchanging; thus it is relatively simple to interpret the directionality of dichromatism gains and losses. Regardless, monochromatic blackbird species tend to have female song and we found a similar (although statistically unsupported) trend in warbler species with common female song.

CONCLUSIONS

Among Parulid warblers, female song is not correlated with migratory status, melanin or carotenoid dichromatism, even though migration and plumage dichromatism are correlated with each other. Nearly all species that duet are sedentary and monochromatic, but the presence of dichromatic and migratory species with female song prevent any correlation of losses of song with losses of migration. This result counters our predictions based on similar studies and suggests that gains of female song may evolve due to different selective pressures than losses of female song. In contrast, the correlated evolution of migration and plumage dichromatism may indicate that coloration in this group evolves following many of the same pressures that other species (notably the *Icterid* blackbirds) face when adopting a migratory or sedentary strategy. Additionally, the different prevalence of female song in these two families may simply reflect the fact that blackbirds gained female song early in their radiation and warblers did not. If gains and losses of female song occur at different rates, then ancestral condition can set clades on very distinct evolutionary trajectories.

AUTHOR CONTRIBUTIONS

NN and LB wrote the paper and scored plumage characters. NN scored song and migration characters and ran analyses in Mesquite.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: http://journal.frontiersin.org/article/10.3389/fevo.2015.00139

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Territoriality, Social Bonds, and the Evolution of Communal Signaling in Birds

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Communal signaling—wherein males and females collaborate to produce joint visual or acoustic displays—is perhaps the most complex and least understood form of communication in social animals. Although many communal signals appear to mediate competitive interactions within and between coalitions of individuals, previous studies have highlighted a confusing array of social and environmental factors that may explain the evolution of these displays, and we still lack the global synthesis needed to understand why communal signals are distributed so unevenly across large taxonomic and geographic scales. Here, we use Bayesian phylogenetic models to test whether acoustic communal signals (duets and choruses) are explained by a range of life-history and environmental variables across 10328 bird species worldwide. We estimate that duets and choruses occur in 1830 (18%) species in our sample and are thus considerably more widespread than previously thought. We then show that global patterns in duetting and chorusing, including evolutionary transitions between communal signaling and solo signaling, are not explained by latitude, migration, climate, or habitat, and only weakly correlated with cooperative breeding. Instead, they are most strongly associated with year-round territoriality, typically in conjunction with stable social bonds. Our results suggest that the evolution of communal signals is associated with the coordinated defense of ecological resources by stable coalitions of males and females, and that other widely reported associations are largely by-products of this underlying trend.

Keywords: birdsong, chorusing, cooperation, duetting, ecological competition, resource defense, sociality

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INTRODUCTION

Communal signals are joint visual or acoustic displays produced when two or more individuals coordinate their signaling behavior, a communication strategy widespread in social animals, including crustaceans (Tóth and Duffy, 2005), primates (Müller and Anzenberger, 2002), and birds (Hall, 2009). A key feature of these signals is that male and female animals both contribute to a combined signal, often with extreme temporal precision, as in the case of many avian duets and choruses (Mann et al., 2006; Hall and Magrath, 2007). Duetting and chorusing are perhaps not ancestral, but nonetheless an ancient traits in birds (Logue and Hall, 2014; Odom et al., 2014), and a common feature of species in which both sexes sing (Slater and Mann, 2004). Thus, the question of why birds signal communally is central to understanding the factors driving song evolution in females and maintaining song as a trait in both sexes.

Numerous hypotheses have been proposed for the function of communal signals (Hall, 2009), generally based on the concept of competition for either ecological resources, or mates and mating opportunities (Farabaugh, 1982). Ecological hypotheses highlight the importance of cooperative defense of home ranges and foraging territories, with duets and choruses signaling the relative competitive ability of coalitions in terms of quality, stability, or numerical advantage (McComb et al., 1994; Seddon and Tobias, 2003; Radford and du Plessis, 2004; Hall and Magrath, 2007). Social hypotheses focus instead on pair or group bonds, suggesting a role for communal signals in guarding against extra-pair or extra-group fertilization (Sonnenschein and Reyer, 1983; Seddon and Tobias, 2006; Tobias and Seddon, 2009), defending positions in partnerships or groups (Rogers et al., 2007) or signaling commitment between breeding partners (Wickler, 1980). Of course, these factors are not mutually exclusive as territorial behavior and social bonding have partially overlapping functions, perhaps explaining why previous studies have found mixed support for both sets of ideas (Hall, 2000; Marshall-Ball et al., 2006; Rogers et al., 2007; Mennill and Vehrencamp, 2008).

Another obstacle to disentangling the drivers of communal signal evolution is that several alternative proximate explanations have been proposed. For example, duets and choruses are more often reported in the tropics, and thus may simply be associated with latitude, temperature, or climatic stability (Slater and Mann, 2004; Logue and Hall, 2014). Similarly, communal signals are often a feature of social and sedentary species, perhaps because they are a by-product of cooperative breeding (Seddon and Tobias, 2003; Radford and du Plessis, 2004), or otherwise associated with long-term monogamy (Benedict, 2008) and stable social bonds in general (Logue and Hall, 2014). The same pattern viewed in reverse may explain the rarity of duetting in migratory lineages, where social bonds are more likely to break down (Logue and Hall, 2014). Habitat may also play a role, particularly as pair or group members are thought to maintain contact using acoustic communal signals in habitats where visual signals are ineffective, such as dense forests (Slater, 1997; Slater and Mann, 2004; Mennill and Vehrencamp, 2008).

Many of these social and environmental factors are closely interrelated, making it difficult to interpret experimental results in single species studies (Hall, 2000; Seddon and Tobias, 2006; Rogers et al., 2007; Tobias and Seddon, 2009), and creating a severe challenge for comparative analyses (Benedict, 2008; Logue and Hall, 2014). Furthermore, previous analyses have only used incomplete sets of predictors related to key hypotheses, with poor coverage of territorial behavior and social bonds. Thus, our understanding of communal signaling remains patchy and the extent to which we can generalize from previous results is unclear (Odom et al., 2015).

We addressed these issues by compiling information on territoriality, sociality, and the occurrence of duets and choruses across 10328 bird species (99% of extant species richness; see Appendix B in Supplementary Material). For each species, we estimated the standard duration of territory defense and social bonds, as well as the density of their primary habitat. All species were scored for migration and cooperative breeding, and we also used geographical range polygons to quantify environmental predictors, including latitude and climatic stability. These datasets offer a useful perspective on the relative roles of social and environmental factors in regulating song evolution in both sexes, particularly as the taxonomic sampling is so comprehensive.

Most studies investigating the function of communal signaling in birds have focused either on single species or single clades (e.g., Odom et al., 2015). The most extensive analyses to date have dealt with a regional passerine avifauna (300 North American species; Benedict, 2008), or subsets (<5 %) of the global avifauna with high quality data (Logue and Hall, 2014). While these studies have highlighted potential mechanisms, they are sensitive to regional or taxonomic biases in the availability or quality of data, along with other sampling effects (e.g., tropical species are absent from the North American sample). Our approach is designed to assess general patterns while minimizing sampling effects by revising and updating data from all birds, the largest terrestrial vertebrate radiation. We further account for variation in data quality by assigning all species to one of four categories of uncertainty (see Materials and Methods).

We conducted two analyses. First, we combined all intrinsic and extrinsic traits, along with the evolutionary relationships among lineages, into a Bayesian mixed model (Hadfield, 2010; Hadfield and Nakagawa, 2010). This method can be used to identify key correlations but is much less informative about the sequence of evolutionary events giving rise to duets and choruses over time. Thus, we used a second Bayesian analysis of correlated evolution (Pagel and Meade, 2006) to estimate transition rates to and from communal signaling under a range of different character states. We used these analyses to clarify the extent to which ecological, social, and environmental drivers provide the most general explanation for global patterns in communal signaling, and whether the evolution of intrinsic life history traits facilitates the evolution of communal signaling (or vice versa).

MATERIALS AND METHODS

Definitions

We define communal signaling as an acoustic display involving two or more members of a social unit, including both males and females. Their contribution to the display must include long-range acoustic signals that are coordinated or stereotyped in some way, whether they be loosely synchronous, regularly alternating, or precisely interwoven. In many cases, the primary long-range acoustic signal in birds is termed the "song," but because we are interested in the underlying processes giving rise to communal signals, our definition extends beyond songs to include other long-range vocalizations with song-like functions, including non-vocal signals. Thus, for example, coordinated calling by pair-members in some seabirds is considered duetting (Bretagnolle, 1996), as is joint drumming by male and female woodpeckers (*Picidae*). Our definition includes all classic duets and choruses (Farabaugh, 1982), but excludes various multi-individual vocalizations, including contact calls, alarm calls, and flight calls. Further, details on discriminating these categories are given below.

In line with convention, we defined duets as communal signals involving two individuals, whereas choruses involve three or more individuals. Following previous studies (e.g., Logue and Hall, 2014), we pooled duetting and chorusing species together in our main analyses because (1) the division between them is very imprecise with many chorusing species occasionally duetting, and vice versa, causing much confusion in the literature, and (2) both forms of behavior are likely to evolve through similar mechanisms (Seddon, 2002; Seddon and Tobias, 2003).

We also collated data on key intrinsic and extrinsic factors hypothesized to play a role in the evolution of communal signaling (Hall, 2009). Specifically, following previous studies (Jetz and Rubenstein, 2011; Salisbury et al., 2012; Pigot and Tobias, 2015), we classified species according to their degree of territoriality (non-territorial, weakly territorial, or year-round territorial); social bond stability (solitary, short-term bond, or long-term bond); type of mating system (cooperative or non-cooperative); movement (non-migratory, partially migratory, or migratory); and habitat (open, semi-open, and dense).

We defined year-round territoriality as territory defense lasting throughout the year rather than residency within a restricted area. For example, species that are vocal and aggressive (responsive to playbacks) for part of the year, and then remain in the same general area silently and unobtrusively for the rest of the year, are classified as seasonally rather than year-round territorial. We defined long-term social bonds as pair or group bonds extending beyond a single year, although this does not necessarily imply that they are year-round. Thus, migratory species in which pair members habitually reunite in subsequent breeding seasons are treated as having long-term social bonds. A detailed rationale, with explanation of our assignment of species to all these categories, is given in Appendix A of Supplementary Material, and summarized in **Table 1**.

Data Collection

We compiled data from field observations, feedback from regional experts, published literature, sound archives, and other online sources of information. Details of signaling behavior, social system, territorial behavior, and movements in birds were compiled in a global database through direct observations by JAT and NS. Observations of >4000 breeding bird species spanned a 20-year period including fieldwork in Europe, the Middle East and North Africa, sub-Saharan Africa, Madagascar, South-east Asia, Australasia and the South Pacific, and extensively in North, Central and South America, with a focus on female song, duetting behavior, social systems and year-round movements (see, e.g., Tobias and Williams, 1996; Tobias and Seddon, 2000, 2003a,b; Seddon et al., 2002, 2003; Tobias, 2003b; Tobias et al., 2008, 2011). Throughout, playbacks were routinely used to assess the strength and seasonality of territory defense, and the contribution of males and females to territorial interactions, at different seasons when possible. To augment these observations, we solicited feedback from field biologists and ornithologists with experience of particular regions or clades. This included a number of professional birding guides who together observe >5000 bird species per annum, often using playback to show rare species to clients.

In addition to information generated from fieldwork, we conducted a thorough review of published literature and online resources. A major source of information was *The Handbook of the Birds of the World* (HBW) series (del Hoyo et al., 1992-2013), comprising 16 edited volumes of species and family accounts for all known bird species. The referenced species accounts, including sections dedicated to vocalizations and movements, are compiled by experts in focal taxa. This information was supplemented by family monographs, regional handbooks and key digital resources, such as Birds of America Online. We also conducted extensive searches for information on communal signaling through primary electronic databases (e.g., *ISI Web of Knowledge, Scopus*), and the meta-search engine *Google Scholar*. We used standardized search terms to identify sources of information for communal signaling (birds, cooperative signal*/song/singing, communal signal*/song/singing, chorus, chorusing, duet, duetting), territoriality (birds, territor*, year-round territor*, long-term territor*, stable territor*, breeding territor*, flock territor*, non-territor*), and social bonding (birds, social bond*, pair bond*, group bond*, mate retention, mate fidelity, monogamy, divorce; where asterisks (*) denote multiple possible suffixes). Relevant secondary articles were identified from the references cited in books or articles found using these approaches.

Finally, we extended recent efforts (Logue and Hall, 2014) to extract information from sound recordings, images, and videos stored in public and private sound archives to compile evidence of communal signaling, with a focus on Macaulay Library (http://www.macaulaylibrary.org), Xeno-canto (http://www.xeno-canto.org), and the Internet Bird Collection (ibc.lynxeds.com). Together, these sources contain material for almost all the world's birds. We listened to sound files to verify putative cases of communal signaling and to survey signaling behavior in poorly known species. In addition, we checked accompanying metadata compiled by field recordists for textual confirmation of communal signaling and other details.

Details of methods for assigning species to categories of territory and social bond duration are provided in Appendix A of Supplementary Material. We classified signals as communal with due caution, bearing in mind several alternative possibilities. Distinguishing other multi-individual signals (e.g., contact calls, flock calls, alarm calls, flight calls) was generally straightforward, both in the field and using sound recordings, because these types of acoustic signal are relatively simple, uncoordinated among individuals, and often phylogenetically conserved variations on a theme. We interpreted multi-individual acoustic signals as evidence of communal signaling when they were coordinated or stereotyped. Typical examples include call-and-answer duets, where the gap between songs is shorter and more consistent than in contests between territorial rivals. Similarly, concurrent bursts of acoustic signals from multiple individuals in group-living species often provided a distinctive signature of communal

TABLE 1 | Definitions of response and predictor variables used in statistical analyses.

Variables	Description and categorization
Communal signaling	(1) Non-communal signallers: a species that does not sing communally, only ever as single individuals
	(2) Communal signallers: a species that produces acoustic signals in groups, either as a duet or chorus
Territoriality	(1) Non-territorial: species that never defend territories, including those that defend very small areas around nest sites, including seabirds, or species where males defend song or display posts only
	(2) Seasonal or weak territoriality: species with broadly overlapping home ranges, or that habitually join mixed flocks with poorly defined spatial ranges (even those that may defend their position in such flocks)
	(3) Year-round territoriality: species that defend territories all year, including migrants that are territorial on both the breeding and non-breeding grounds
Social bonds	(1) Solitary: species that does not form pairs, or forms them only briefly during the courtship phase (uniparental care)
	(2) Short-term pair/group bonds: species with seasonal consorting of pair/group members AND unstable pair/group composition in subsequent breeding attempts [low mate fidelity, high divorce rate (>50% per annum)]
	(3) Long-term pair/group bonds: species with year-round consorting of pair/group members, or those with seasonal pair/group bonds AND stable pair/group composition in subsequent breeding attempts [high mate fidelity, low divorce rate (<50% per annum)]
Cooperative breeding	(1) Non-cooperative: a species in which no more than two birds, the parents, care for the young
	(2) Cooperative: a species in which more than two birds habitually care for the young
Migration	(1) Sedentary: does not migrate
	(2) Partially migratory: minority of population migrates long distances, or most of population undergoes short-distance migration, nomadic movements, distinct altitudinal migration, etc
	(3) Migratory: majority of population undertakes long-distance migration
Habitat	(1) Open: species primarily occurs in desert, grassland, open water, low shrubs, rocky habitats, seashores, and cities
	(2) Semi-open: species primarily lives in open shrub land, scattered bushes, parkland, dry or deciduous forest, thorn forest
	(3) Closed: species primarily lives in tall evergreen forest with a closed canopy, or in the lower vegetation strata of dense thickets, shrubland or marshland
Latitude	Median midpoint latitude as calculated from global range polygons
Mean annual temperature	WorldClim Index BIO1 (C°) averaged across the 1° grid cells corresponding to the breeding distributions defined by global range polygons
Temperature range	WorldClim Index BIO7 (C°) averaged across the 1° grid cells corresponding to the breeding distributions defined by global range polygons
Annual precipitation	WorldClim Index BIO12 (mm) averaged across the 1° grid cells corresponding to the breeding distributions defined by global range polygons
Precipitation range	Difference between WorldClim Index BIO13 and BIO14 (mm), the precipitation of the wettest and driest months respectively, averaged across the 1° grid cells corresponding to the breeding distributions defined by global range polygons

signaling. In practice, assignment to categories was often simplified by the behavior al context of signaling, either directly observed in the field, or reported in sound file metadata. When the context was unclear, we did not necessarily assume communal signaling was occurring when two or more individuals were audible producing long-range acoustic signals, as in many cases it was difficult to rule out counter-singing by individuals in neighboring territories (i.e., different social units).

A separate challenge involves confirming that both males and females are contributing to communal signals. However, in practice this problem was largely irrelevant to socially monogamous species where we assume pairs contain one individual of both sexes. Although scoring species for female song can be challenging when the sexes are monomorphic (Odom et al., 2014), communal signaling is often easier to detect and verify in such cases because both pair members signal in unison. Conversely, it is sometimes difficult to be sure that females are contributing to choruses in group-living species. However, both sexes are known to contribute to choruses in color-marked populations of several species, and the same message emerges from observations of many monomorphic group-living species in which all individuals can be observed signaling together. Indeed, after considerable attention to this issue, we are not aware of any case of chorusing species where signaling is limited to one sex.

With respect to duetting species, we revised and updated some previous classifications with new information when it was clear that the original reports were based on misinterpretation. Our survey suggested that some species previously listed as duetters should be delisted for the purpose of our analyses because (1) the evidence for communal signaling clearly involved rare or unusual behavior, and (2) literature reports of communal signaling are sometimes based on different definitions of duets and choruses, with these terms often used loosely. For example, some authors use the terms “duet” or “chorus” to refer to counter-calling between neighboring territorial males, or to acoustic signals used in alarm and agitation. One such case is the American Rock-wren *Salpinctes obsoletus*, in which males can produce simple calls during close-quarters agonistic interactions with neighbors, sometimes accompanied by the female, leading to this species being listed as a duetter (Odom et al., 2015). We re-classify the species as a non-duetter because the calls are short-range signals given in agitation, whereas females do not sing or produce any other long-range signals, either alone or with the male. This distinction is important because males and females of all pair- or group-living bird species occasionally produce short-range acoustic signals at the same time. Counting all such cases as duets will obscure the underlying distribution of conventional duetting, potentially biasing the results of comparative studies, as recently shown in a similar dataset for avian cooperative breeding (Griesser and Suzuki, 2016). Where switches in classification were less certain, we simply assigned a lower score for data quality (see below).

Classifying the world's birds to behavioral and life history categories is challenging, not least because direct information is scarce for many species. We also acknowledge that the boundary between categorical variables is unavoidably blurred, making assignments subjective in some cases. For example, it can be difficult to judge whether a poorly known tropical species is territorial year-round or only during the breeding season, or whether its social bonds endure for a short breeding season or for multiple years (Stutchbury and Morton, 2001). However, for most species there is a growing body of information about local movements and whether particular pairs/groups remain spatially fixed over time. We followed simple rules-of-thumb to classify all cases. When evidence suggested that pairs or groups are highly sedentary, we assumed that pair/group bonds lasted more than a year on average (<50% divorce rate per annum). Our assumption is based on the general pattern established in field studies of color-marked bird populations: we are not aware of any bird species which lives in sedentary pairs or groups year-round and which also has a >50% annual divorce rate. When pair/group bonds break down seasonally (e.g., in migratory species, waterbirds), the uncertainty over divorce rates increases. We assumed that bonds lasted <1 year on average (i.e., >50% pairs or groups that reform in subsequent seasons contain new combinations of individuals) when there is evidence of this outcome in phylogenetically or ecologically related lineages (e.g., many migrant passerines). Conversely, when there was strong evidence that monogamous pairings extended beyond a single breeding season in phylogenetically or ecologically related lineages (e.g., seabirds and many migrant non-passerines), we assumed that >50% pairs reforming over subsequent seasons were likely to contain the same individuals. These procedures may result in some degree of misclassification but we argue that our categories provide an accurate general reflection of variation in social bond duration across the world's birds. Moreover, uncertain cases are reflected in scores of data quality and thus our conservative analyses are restricted to more objective cases.

Finally, assignment to categories may be uncertain when information is drawn from a single locality, or when species vary in a particular trait across their range. Whenever possible, we selected categories on the basis of their predominance in terms of behavior, or their prevalence across the global range of a species. Thus, for example, we classified species as year-round territorial only if such populations made up more than 50% of the global breeding range. In variable or wide-ranging species, we ensured that predictors and response variables were drawn from the same (or geographically closest) population.

Spatial and Climatic Data

Because communal signaling and the underlying degree of cooperation among individuals may be influenced by latitude and climatic conditions (Rubenstein and Lovette, 2007; Jetz and Rubenstein, 2011; Odom et al., 2014), we used the geographical range polygon for each species to extract median midpoint latitude and environmental data (mean annual temperature, temperature range, annual precipitation, and precipitation range) from the Worldclim database (http://www.worldclim.org), following standard methods (Pigot et al., 2010). Species lacking adequate data were excluded for the relevant analyses, leaving a sample of 9230 species for nested taxonomic models. After further excluding species for which no published genetic data yet exist, we retained a sample of 5505 species for phylogenetic mixed models.

For further details of hypotheses and data collection methods, see Appendix A in Supplementary Material; for a complete list of species and sources of information, see Appendices B, C in Supplementary Material.

Data Limitations, Inference, and Uncertainty

In this study, we provide the first global assessment of communal signaling, territoriality, and social bond duration across the world's birds. The scale of this assessment raises a number of challenges, not least because a large proportion of bird species remain poorly known. Nonetheless, we argue that sufficient information is now available to assign almost all species to a useful classification system. To achieve this goal, we used multiple strands of evidence, including direct observations and extensive unpublished information from sound archives and expert field ornithologists. Given the rapid pace of recent ornithological exploration in remote regions, most bird species–aside from a handful of extreme rarities–are now familiar to fieldworkers or birding guides at particular localities where information gathered on repeated visits can provide insight into territorial and social behavior through time. This influx of information is not readily available in published literature, but allows many species previously considered data deficient to be categorized with greater confidence. For example, *Cacicus koepckeae* is excluded from previous literature-based analyses of communal signaling (Odom et al., 2015) but included here as a territorial duetting species on the basis of field observations (Tobias, 2003a) and sound files archived online (see http://www.xeno-canto.org/species/Cacicus-koepckeae).

Where evidence was inconclusive, classifications were inferred partly from information relating to multiple close relatives, following standard procedures (Wilman et al., 2014). For communal signals, this type of inference was only used when there were strong grounds for doing so–for instance, when behavior was consistent across close relatives, backed up by circumstantial evidence such as field reports, sound recordings or videos. A similar approach was taken for life history attributes, with estimates of the duration of territory defense or social bonds often representing a best-guess when sufficient evidence was available from field observations, literature, and related species (see Appendix A in Supplementary Material for full details and rationale). Inferences were never drawn on the basis of phylogenetic relationships alone. Nonetheless, given the scale of our dataset, some lineages are almost certainly misclassified. A detailed summary of possible sources of error is provided in Appendix A of Supplementary Material.

To provide more information about variation in uncertainty, we assigned classifications of all species to four categories of data quality: A, high quality data based on published sources or strongly supported evidence from direct observations; B, medium quality data, including cases where the classification is very likely correct but largely based on field observations and reports; C, low quality data based on few observations, or unsubstantiated literature reports; D, absence of direct evidence. Henceforth, we refer to A as the conservative dataset, B as the medium quality dataset, and C and D together as poor quality data. The degree of inference from congeners is reflected in these categories, from very low inference in A, and minor, supporting inference in B, to larger levels of inference in C. Classifications of data-deficient species (D) were entirely based on inference. Where we found a strong consensus from all strands of evidence, we scored data quality higher than where evidence was in conflict. For example, golden whistlers *Pachycephala pectoralis* are reported to duet in captivity (Brown and Brown, 1994), but this behavior has not been detected in the field. Although this report may use a different definition of duetting to that employed in this study, it nonetheless increases the level of doubt about the lack of duetting observed in congeners, and thus we score most other *Pachycephala* species with an increased level of uncertainty. Finally, because levels of uncertainty often differ for information on communal signaling and general ecology, we scored data quality for both signaling and ecological data separately. Inclusion of species in analyses depended on both signaling and ecological data meeting minimum standards. Species were included (1) in our taxonomic analyses only if they scored A/B for song data quality and A/B/C for life history data quality; (2) in our main (medium quality data) analyses only if they were scored A for song data quality, and A/B for life history data quality; and (3) in our conservative analyses only if they scored A for both signaling and life history data quality. Like all datasets of global scale, ours will undoubtedly benefit from further quality control and curation, and we hope to facilitate this process by archiving all data online in association with this article.

Comparative Analyses

Our analyses included a range of (categorical) behavior and life history variables, and (continuous) climatic variables extracted from geographical ranges. We assessed the effects of these factors on the occurrence and evolution of communal signaling using Bayesian binary-response mixed-effect models with logit link, implemented in the R package, *MCMCglmm* (Hadfield, 2010; Hadfield and Nakagawa, 2010). To account for the potential effects of phylogenetic inertia, we adopted two complementary modeling approaches: (1) Bayesian taxonomic mixed models (BTMM) in which Order, Family and Genus were entered as nested random factors for all species, and (2) Bayesian phylogenetic mixed models (BPMM), in which phylogenetic relationships were entered as a random factor, assuming a Brownian model of evolution. This random term translates into phylogenetic variance equivalent to Pagel's λ (Pagel, 1999). We included BTMM as this allowed us to include all species with sufficient data ($n = 9230$), whereas BPMM were run on trees obtained from a published multilocus phylogeny, pruned to species with molecular data ($n = 5505$ for the medium dataset; and $n = 1665$ for the conservative dataset; Jetz et al., 2012). We first performed models (BTMM/BPMM) with 11 predictors (10 input variables: 4 intrinsic/life-history and 6 extrinsic/environmental; Table S2). We then re-ran the same models including significant predictors (i.e., those that were statistically significant in both full models) and their second-order interactions. Only interactions with strong effects were included, following (Gelman and Hill, 2007) (see electronic Supplementary Material, Table S3).

For all BTMMs and BPMMs, we used a Gelman prior for random effects (in *MCMCglmm* (Hadfield, 2010) using the command "gelman.prior" (Gelman et al., 2008) with $V = 10^{-6}$, $nu = -1$). We ran three independent runs of *MCMCglmm* for all models models, each run for 1.5×10^6 iterations. After discarding a burn-in of 10^6 and a thinning of 5000, the remaining 1000 samples constituted our posterior distribution for each chain. We checked convergence of model parameters (fixed effects and random effects) using the Gelman-Rubin statistic (the potential scale reduction, PSR, factor should be <1.1 among chains (Gelman and Rubin, 1992); all PSR factors met this criterion). We only used posterior distributions from the first of three chains for reporting our parameter estimates (models and 95% credible limits, CLs). Note that in binary models (BTMM/BPMM) a dispersion parameter (akin to residual variance) is unidentifiable (zero). To run the models in *MCMCglmm*, we fixed the parameter > 0, but then rescaled estimates in the results tables (**Table 1**, and electronic Supplementary Material Table S1) so that the parameter $= 0$.

Regression analysis such as BTMM or BPMM are informative about the ecological and social conditions favoring the evolution of communal signaling, but not about the direction of causality. To address this question, we used Pagel's Discrete algorithm implemented in BayesTraits (Pagel and Meade, 2006) to test whether and how key traits have evolved in tandem across the same phylogenetic tree described above. We defined key traits as those significantly correlated with communal signaling in mixed models (BTMM and BPMM). The sample size ($n = 5669$ species) is slightly larger than for BPMMs because fewer species lacked relevant variables. The BayesTraits method uses a likelihood ratio test to compare a model in which the traits evolve independently (independent model) with one in which they evolve in tandem (dependent model). It also estimates the likelihood of evolutionary transitions among traits, assuming correlated evolution. These transition rates provide information about the relative stability of communal signaling with or without a particular life-history trait (and vice versa).

We used this approach to model how communal signaling was associated with territoriality, social bonds and mating system (independent and dependent models in each case, 6 models in total). As the method can only be applied to binary traits, we dichotomized variables initially classified into three categories (see Table S1). We grouped territoriality into: 1 = species with year-round territoriality, 0 = weak/seasonal territoriality or non-territorial. Similarly, we dichotomized social bond duration into: 1 = long-term (>1 year) pair/group bonds, 0 = short-term pair/group bonds or non-sociality. We grouped traits in this way for two main reasons. First, it produces the most balanced sampling in a dichotomous framework because relatively few species are non-territorial or lack social bonds (**Figure 3**). Second, this division most closely reflects existing hypotheses for communal signaling, which point to the importance of year-round territoriality (Benedict, 2008) and social stability (Logue and Hall, 2014).

We ran each BayesTraits model for 1.1×10^7 iterations, discarding an initial burn-in of 10^6 and sampling the chain every 10,000 iterations, resulting in a sample of 1000 per model/per tree. We ran two independent chains on each tree in the sample and combined samples resulting from all the runs, which constituted our posterior distributions for all parameter estimates. In all cases, a hyper prior of an exponential distribution (seeding from a uniform distribution on the interval 0–100) for a reversible jump procedure (see http://www.evolution.rdg.ac.uk/BayesTraits.html). The trees were scaled by 0.05, as the rates are proportional to the branch lengths. This places the transition rates on a more usable scale and does not alter their relative values. For each chain, the marginal likelihood was calculated using a stepping stone sampler (Xie et al., 2011): 100 stones were distributed according to a beta distribution (shape 0.400000, scale 1.000000) and each stone was run for 25,000 iterations.

RESULTS

Prevalence and Distribution of Communal Signaling

We found evidence of communal signaling in 1830 species (18%) in the total list of 10328 species (see Appendix A in Supplementary Material). Excluding species with poor signaling data (category C and D) produced a smaller total of 1812 species with communal signaling (17%); of these, duetting occurs in 1627 (∼16 %) species, a total that includes chorusing species which occasionally duet. Duetting was previously thought to be present in only 222 (or ∼2–3%) of species (Thorpe, 1972; Kunkel, 1974; Farabaugh, 1982; Hall, 2004), with the estimate recently revised to 420 species (∼4%; Hall, 2009). Even excluding species with poor quality data (categories C and D), our results indicate that communal signaling is taxonomically widespread, evolving multiple times across the avian tree of life (**Figure 1**), occurring in 26/39 orders (67%) and 110/225 families (49%), with roughly equal prevalence in the passerines (1102/6049, 18%) and non-passerines (710/3522, 20%).

Our data confirmed that the geographical distribution of communal signaling is uneven, with greatest prevalence in western Amazonia, western and central Africa, Indo-Malaya, and northern Australia (**Figure 2A**). This distribution remains essentially unchanged when focusing on duetting species (**Figure 2B**) and conservative data (Figure S2). In general, more duetting and chorusing species occur in the tropics (**Figures 2**, **3A**). However, this pattern is largely driven by greater species richness in the tropics, and after correcting for the gradient in overall diversity we find that communal signaling peaks in the southern hemisphere (**Figure 3A**). Across the world's terrestrial biomes (Olson et al., 2001), the highest proportions of species with communal signaling (18–20%) occur in tropical and subtropical habitats (grasslands, savannas, shrublands, and both moist and dry broadleaf forests), while the smallest proportions (∼6%) occur in tundra and boreal forests (Table S1).

Predictors of Communal Signaling

We found that there is a strong phylogenetic signal in the occurrence of duetting and chorusing (**Figure 1**), with evolutionary history a dominant predictor of these traits in our combined full (Table S2), and final models (Table S3). In the BTMM, taxonomy (Order, Family, Genus) explained 16–39% of the variance in communal acoustic signaling, and in the BPMM, phylogeny explained ∼96% of variance (at both levels of data certainty we used in analyses; see below). This result is not surprising given that communal signaling is widespread in some clades (e.g., antbirds *Thamnophilidae*) but absent in others (e.g., hummingbirds *Trochilidae*). However, the strength of phylogenetic signal may be inflated because we sometimes inferred shared character states among close relatives. We note that (1) even a much weaker phylogenetic signal supports our assumption of a Brownian motion model of evolution in subsequent analyses, and (2) inference of shared character states among relatives does not affect our main results because we use both taxonomic (BTMM) and phylogenetic (BPMM) models to correct for phylogenetic non-independence when testing for associations with communal signaling.

We found that territoriality, social bonds, cooperative breeding, latitude, and temperature range were all significant predictors of communal signaling in BTMMs (Tables S2, S3). No such association was found between habitat density or migration and communal signaling. However, the results of this hierarchical model should be treated with some caution because the BTMM contains only basic evolutionary information and may therefore fail to account adequately for phylogenetic non-independence (pseudoreplication).

Table S4 When we re-analyzed our data using BPMM, thus controlling for phylogeny, we found that communal signaling was significantly associated with territoriality and social bond stability, and that cooperative breeding was the only other significant (but weaker) correlate. We note that territoriality and cooperative breeding are strongly correlated: a model predicting cooperative breeding as a function of territoriality has an overall estimated R^2 of 0.956, with an estimated partial R^2 of phylogeny of 0.954; the coefficient estimate for the scaled territoriality value is −1.849 (CI: −2.677, −1.172). In contrast, we found no evidence that latitude, habitat density, migration, or climatic variability were associated with communal signaling (Tables S2, S3). Thus, although species with duets and choruses appear to be

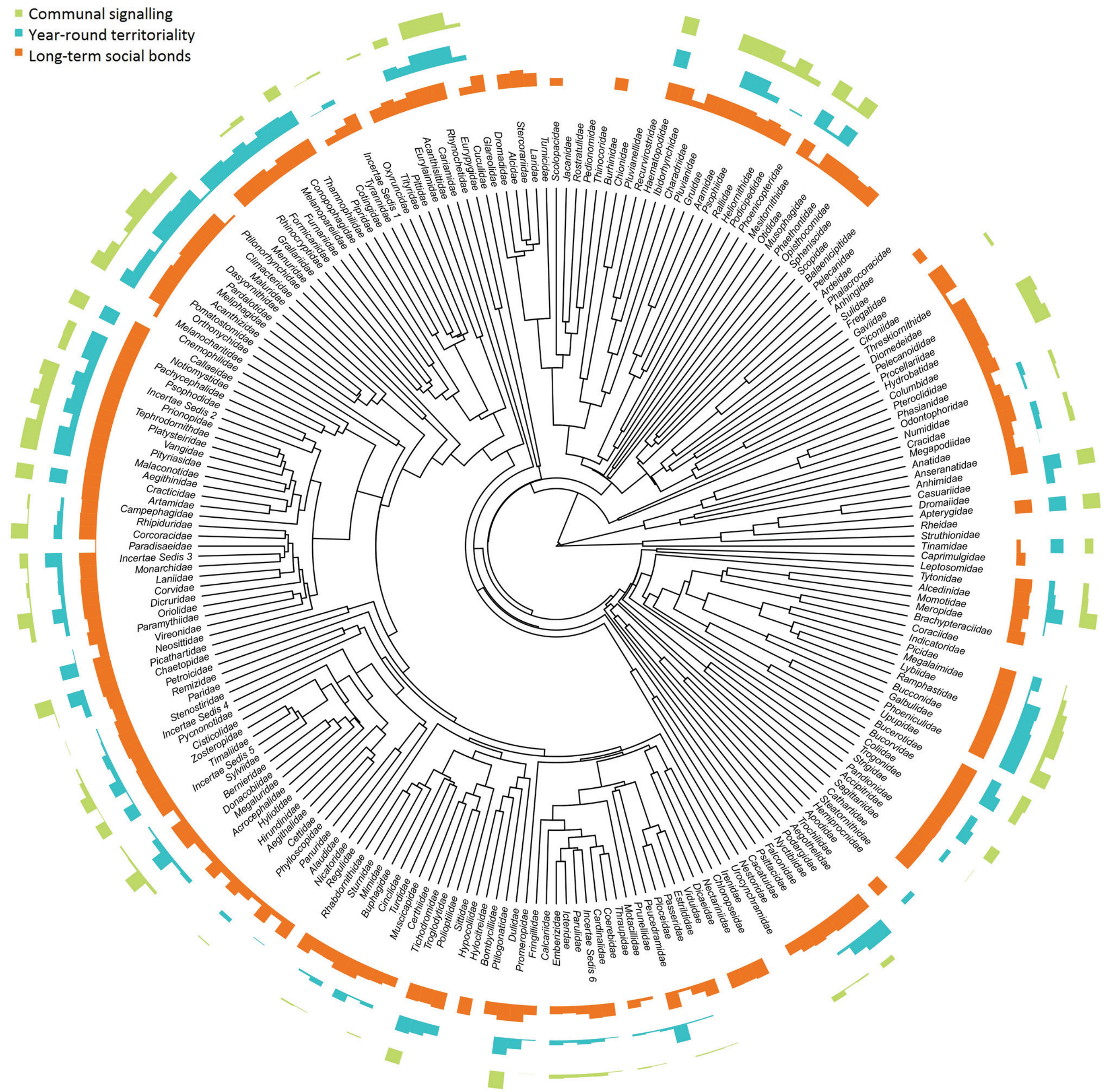

FIGURE 1 | Proportion of species with communal signaling, long-term (>1 year) social bonds, and year-round territoriality across avian families. Data are aggregated from 5505 species within 224 bird families and plotted at the tips of a maximum clade credibility phylogenetic tree. Species with high uncertainty were removed prior to calculating family totals; data presented are therefore the same as our main analyses (medium certainty); patterns based on more conservative data are very similar (see Figure S1). Bars are scaled to the proportion of species in each family expressing a particular trait: tallest bars = all species; shortest bars = zero species.

more prevalent in relatively stable tropical habitats (**Figure 2A**) with low annual variation in temperature (**Figure 3B**) and rainfall (**Figure 3C**), these associations disappeared when we accounted for evolutionary relationships and life-history traits. Running BPMMs on conservative data produced very similar results, except that the relationship between cooperative breeding and communal signaling then becomes non-significant (Table S2).

The fact that year-round territoriality and long-term social stability emerge as the most important predictors of communal signaling seems to make sense because many duetting or chorusing species share both these life history traits (**Figure 4**).

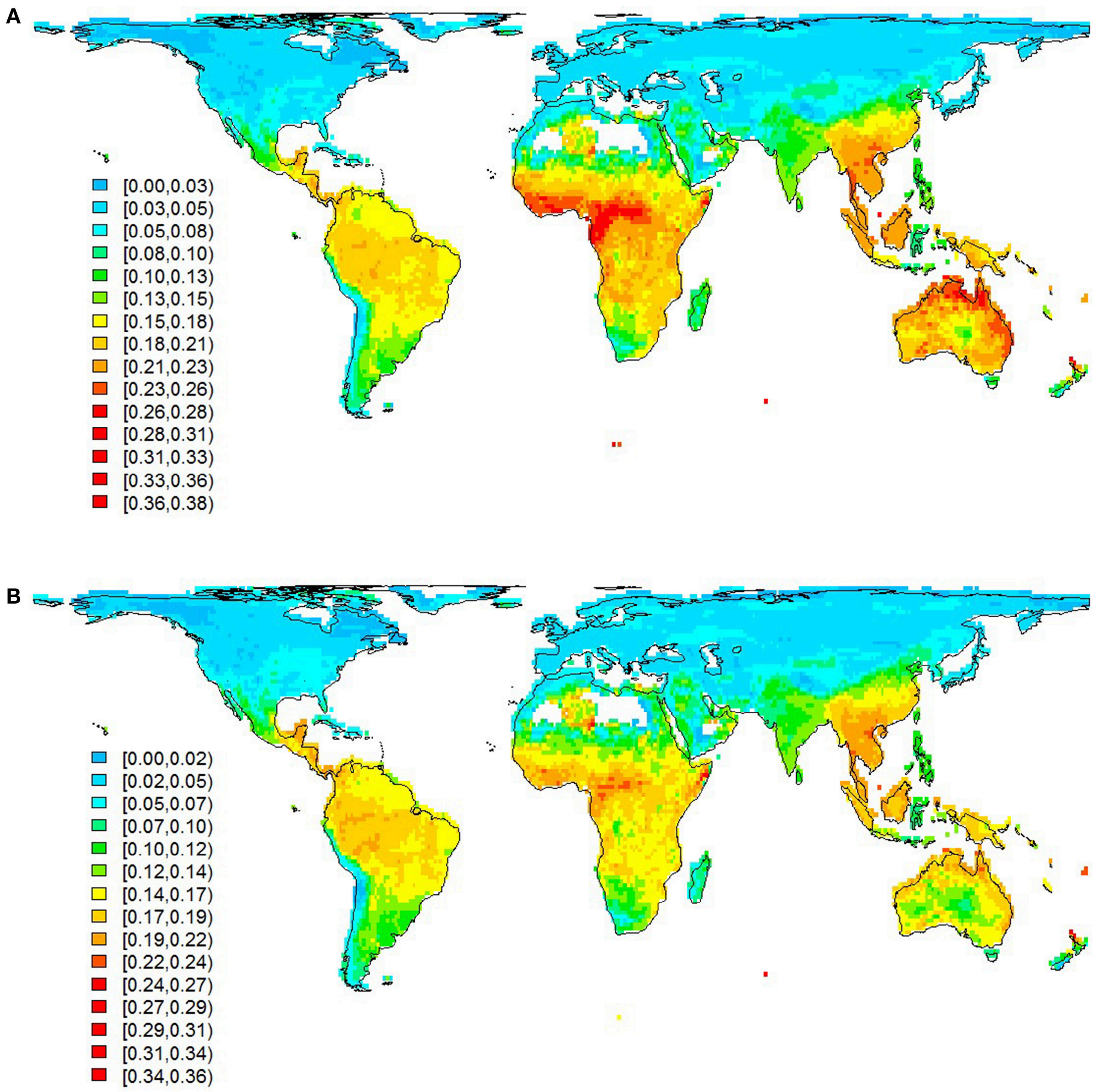

FIGURE 2 | Global patterns in the distribution of communal signaling. Prevalence of species with **(A)** communal signaling and **(B)** duetting (subset of A), calculated as the proportion of total species occurring in 110 × 110 km grid cells. Legend gives lower and upper values for each color. Grid cells with <10 species were removed (e.g., Sahara). Species with high uncertainty were removed prior to calculating community totals; data presented are therefore the same as our main analyses (medium certainty) although a slightly smaller sample size (*n* = 5018) because some species lacked accurate maps; patterns based on more conservative data are very similar (see Figure S2).

However, the underlying correlation between territoriality and sociality is accounted for by our mixed modeling approach, and in any case the relationship between them was highly asymmetric: 3010 species with both strong territoriality and long-term social bonds made up 97% of the 3096 species with strong territoriality, but only 40% of the 7556 species with long-term social bonds. We also detected a significant interaction between territoriality and sociality (Table S3). Specifically, our results suggest that having one or other of year-round territoriality or social stability has a very large effect on the probability of communal signaling, particularly in the case of year-round territoriality, but that it's less important to have both (Table S3).

Co-Evolution of Communal Signaling with Life-History Traits

When we used BayesTraits analyses to examine evolutionary transitions between states, we again found strong evidence that communal signaling evolved together with year-round territoriality (average log Bayes Factor 824.66), stable social bonds (average log Bayes Factor 310.70) and, to a lesser extent, cooperative breeding (average log Bayes Factor 26.23; Tables S4, S5). A log Bayes Factor above two can be viewed as significant (Kass and Raftery, 1995). Re-running these analyses on conservative data produced similar results (Tables S4, S6). The associations were slightly weaker (although still very strong) between communal signaling and both year-round territoriality

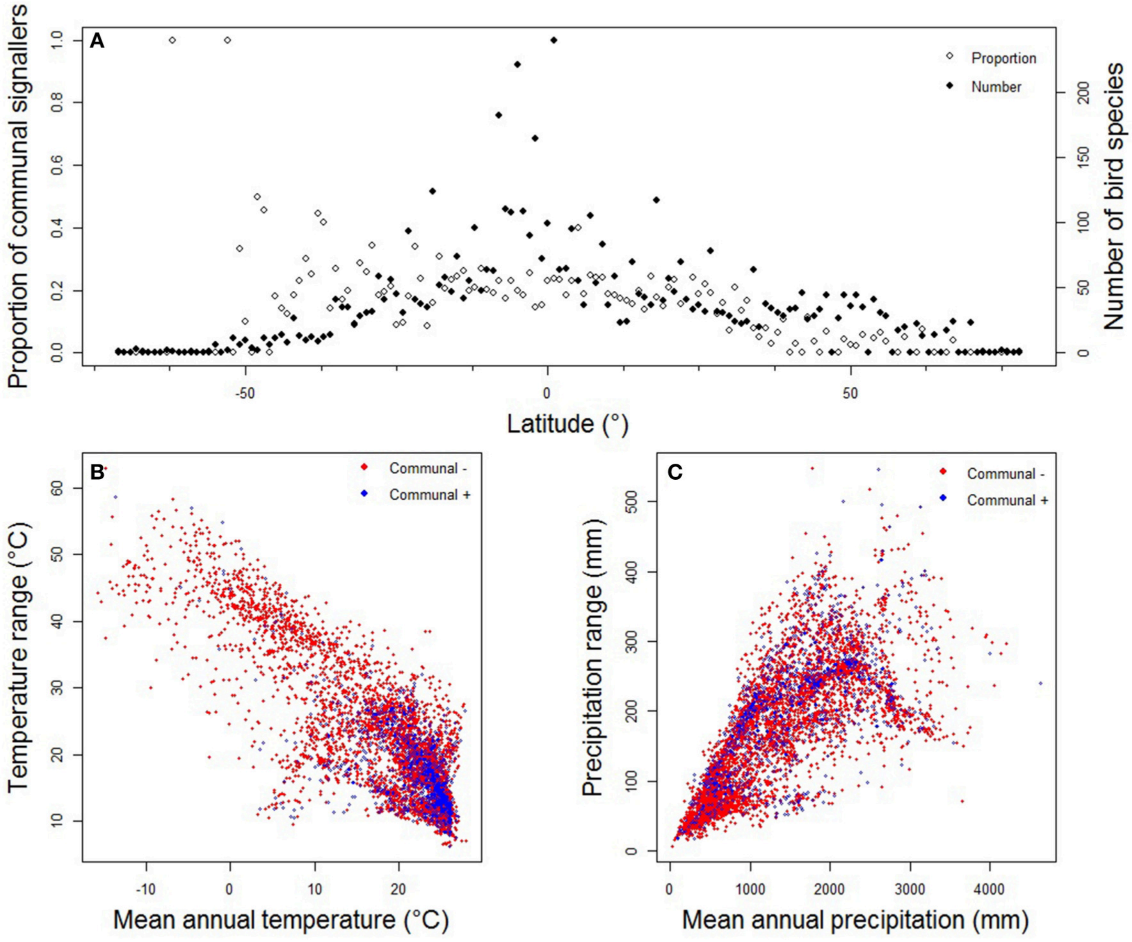

FIGURE 3 | Spatial and environmental correlates of communal signaling in birds. Panels show the relationship between **(A)** communal signaling and midpoint latitude of species geographic ranges; **(B)** communal signaling (= Communal +) and temperature; and **(C)** communal signaling (= Communal +) and precipitation. Points in **(A)** are the proportion or number of species occurring within each 1° band of latitude; points in **(B)** and **(C)** represent data from a single species. Species with high uncertainty were removed and thus data presented are the same as our main analyses (medium certainty; $n = 5505$); patterns based on more conservative data are very similar (see Figure S3).

(average log Bayes Factor 528.69) and stable social bonds (average log Bayes Factor 229.81). However, the significant association between communal signaling and cooperative breeding in the conservative dataset was much lower (average log Bayes Factor 6.29) and not significant in every tree (91 out of 100 had a Bayes Factor > 2).

Figure 5 illustrates the flow between evolutionary states detected in BayesTraits analyses. The arrows depicting this flow provide information about the stability of evolutionary states, with a low transition rate toward and a high transition rate away from a particular state indicating low stability of that state. For example, in (C) State 3 (communal signals and weak social bonds) is highly unstable, readily transitioning to State 1 (solo signals and weak social bonds) or State 4 (communal signals and strong social bonds). Similarly, the co-occurrence of communal signaling with cooperative breeding is unstable, readily transitioning to state 3, where breeding is non-cooperative but signaling is communal (**Figure 5D**). Conversely, in (B), State 4 (communal signals and strong territoriality) is stable, with balanced transitions to and from State 2 (solo signals and strong territoriality), and State 3 (communal signals and weak territoriality). The key points to take from **Figure 5** are that q24 (evolving communal signals with territoriality) occurs 20 times faster than q13 (evolving communal signals without territoriality; **Figure 5B**), and that q34 (evolving communal signals with social bonds) occurs 23 times faster than q12 (evolving social bonds without communal signals; **Figure 5C**; Table S5).

DISCUSSION

Our comparative analyses reveal that avian duets and choruses are significantly linked to both year-round territory defense and long-term social bonds, and only weakly associated with cooperative breeding. Furthermore, once we accounted for these relationships, as well as for shared ancestry, we found no

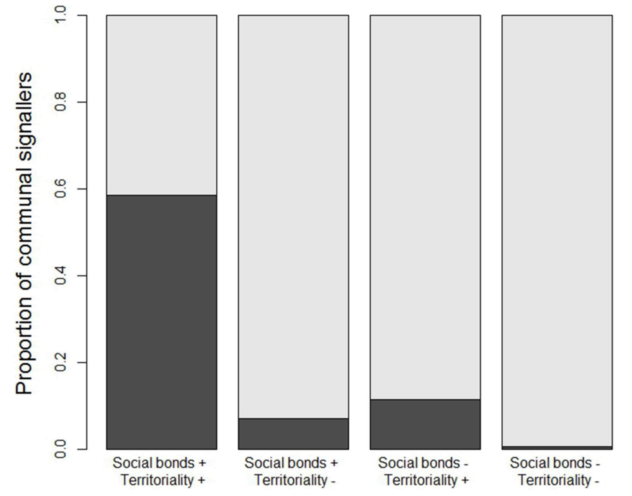

FIGURE 4 | Associations between communal signaling and the stability of territoriality and social bonds. White bars show the proportion of species with communal signaling, black bars show the proportion with non-communal signaling, partitioned among species that have (+) or do not have (–) long-term social bonds and year-round territories (see **Table 1**). High uncertainty data were removed so that patterns are based on the medium certainty data (*n* = 5505 species) used in our main analyses; proportions calculated using conservative data are similar (see Figure S4).

evidence that latitude, climatic variability, habitat, or migration predicted the occurrence of communal signals. These findings are corroborated by patterns of co-evolution among key life-history traits, which indicate that the presence of duets and choruses is most stable in association with territoriality and sociality. Thus, our results suggest that social factors predominate over environmental factors in driving communal signal evolution, and that the intensity and duration of ecological resource defense coupled with social stability provides the most general explanation for communal signal evolution.

The advantage of our broad-scale approach is that it offers sufficient statistical power to compare the effects of multiple factors. Our results shift the emphasis away from previously identified correlations with latitude, habitat density, migration, and climatic variability, perhaps because earlier studies were based on relatively restricted datasets sampled inconsistently across latitudes, climates, or major clades (e.g., passerines vs. non-passerine). This patchy sampling may generate different outcomes because associations vary across clades and contexts. For instance, while it is clear that for some species duets function partly in maintaining contact between pair members in dense habitats (Mennill and Vehrencamp, 2008), many duetting species occur in open environments, implying that habitat density does not provide a general explanation for communal signaling.

By sampling across the full span of environmental and life history variation in the world's birds, we have shown that correlations between communal signaling and environmental (extrinsic) factors are consistently subordinate to correlations with life-history (intrinsic) factors. The importance of species ecology over environmental conditions in promoting communal signaling has not previously been reported, but fits the observation that duets are well known in temperate zone species with year-round territoriality (e.g., tawny owl *Strix aluco*) or long-term social bonds (numerous seabirds), as well as in tropical species with the same underlying traits. Rather than latitude or climate explaining patterns in signaling behavior, our results suggest that the uneven geographical distribution of communal signaling shown in **Figure 2** arises simply because extended forms of territoriality and sociality are biased toward the tropics and southern hemisphere. Indeed, this effect has been reported within evolutionary lineages: in the house wren *Troglodytes aedon* complex, for example, communal signals are common in the tropics where territories are defended year-round, but rare in the temperate zone where territoriality is seasonal (Stutchbury and Morton, 2001).

Selection is likely to favor long-term territoriality and social bonds at low and southern latitudes for a number of reasons (Jankowski et al., 2012; Tobias et al., 2013). First, the climate is generally more stable than in the northern temperate zone (Ghalambor et al., 2006), promoting sedentary lifestyles and stable social bonds. Second, the year-round availability of many ecological resources (Huston and Wolverton, 2009) means that the territories of land-birds are worth defending over longer time-periods. Third, avian populations in the tropics often approach carrying capacity owing to reduced mortality and increased longevity (Wiersma et al., 2007; Williams et al., 2010). Together, these factors place a high premium on the collaborative defense of ecological resources and group membership in the tropics, as territory or group vacancies are theoretically scarce and difficult to regain if lost. In this context, individuals may signal communally to protect their positions in long-term coalitions, which in turn cooperate over signal production to deter rival pairs or groups.

Disentangling the role of territoriality and sociality is challenging because communal signaling frequently occurs in conjunction with both year-round territoriality and long-term social bonds, which often occur together (**Figure 4**). This connection between long-term territoriality and social cohesion suggests that competition for ecological resources increases in parallel with competition over membership of partnerships or coalitions of individuals, perhaps helping to explain why avian duets appear to mediate both cooperation (i.e., joint territory defense; Seddon and Tobias, 2003; Hall and Magrath, 2007) and conflict (i.e., mate- defense; Sonnenschein and Reyer, 1983; Rogers et al., 2007; Tobias and Seddon, 2009). Nonetheless, phylogenetic mixed models revealed that the effect of territoriality was more than twice as strong as that of social bonds (Tables S2, S3), whereas cooperative breeding was only weakly associated, with an effect approximately one quarter that of social bonds.

Similarly, the evidence from evolutionary transitions suggests that the combination of year-round territoriality and communal signaling is a more stable state, and far more likely to co-evolve, than long-term social bonds coupled with communal signaling (**Figure 5**, Table S5). Furthermore, the BayesTraits analyses provide a clue that territoriality may be crucially important as a precursor to communal signaling, whereas long-term social bonds in pairs or groups may actually arise after communal

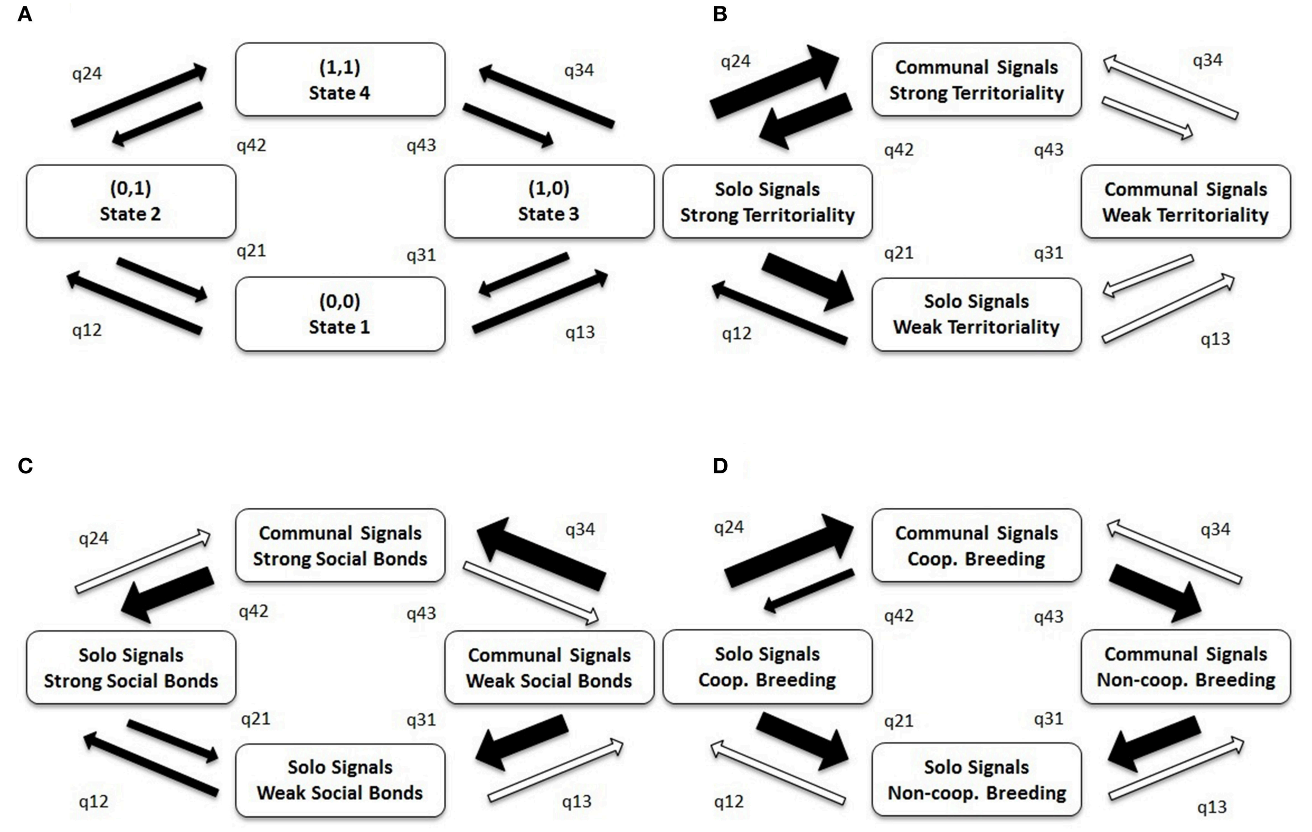

FIGURE 5 | The co-evolution of communal signaling with life-history traits in birds. (A) Model illustrating four possible evolutionary states (1–4) between two traits and eight possible transition paths (q). **(B–D)** Results of BayesTraits analyses testing the relative stability of communal signaling in relation to three other life-history traits: **(B)** territoriality, **(C)** social bonds, and **(D)** cooperative breeding. Strong territoriality = year-round territory defense; Strong social bonds = estimated duration of pair or group bonds >1 year. Broad arrows indicate high transition rates (>40 transitions per lineage per billion years); thin arrows indicate medium transition rates (10–40 transitions per lineage per billion years); open arrows indicate low transition rates (<10 transitions per lineage per billion years); transitions are labeled such that qXY indicates the evolutionary transition from State X to Y. Actual values are provided in Table S5. Species with poor quality trait or phylogenetic data were removed from analyses (leaving a sample of $n = 5669$ species); the results based on conservative data are similar (see Figure S5, Table S6).

signaling evolves–that is, pair and group bonds may result from selection for defending resources as a coalition, rather than vice versa. Although the pattern of evolutionary transitions in our dataset is most consistent with this interpretation, we do not specifically reconstruct ancestral states, and so the question of evolutionary pathways to (and from) communal signaling requires further investigation.

Many cooperatively breeding birds appear to signal as a group, and thus our finding that cooperative breeding is only weakly associated with communal signaling is perhaps surprising. The reason for this outcome becomes clearer when considering the correlation between cooperative breeding and territoriality, which is both strong and largely explained by phylogeny. Of these two associated variables, our results indicate that cooperative breeding is a much weaker predictor of communal signaling, and thus when territoriality is accounted for in phylogenetic models, cooperative breeding has very little additional explanatory power. This is particularly evident in our conservative analyses, where the association between cooperative breeding and communal signaling is removed altogether.

Cooperative breeding is only one form of cooperation in birds, and almost all avian duets and choruses function at least partly in cooperative contexts (Dahlin and Benedict, 2014), suggesting that global patterns of communal signaling can shed light on the evolution of cooperation (Logue and Hall, 2014). In highlighting the importance of long-term social bonds, our findings echo those of previous studies on duetting (Benedict, 2008; Logue and Hall, 2014). Moreover, as pointed out by Logue and Hall (2014), this pattern aligns with theoretical (Trivers, 1971) and empirical studies (Heide and Miner, 1992; Bó, 2005) suggesting that cooperation among individuals is most likely to arise when they associate over prolonged periods. Previous explanations for this effect are mainly based around the concepts of trust, reciprocity or kin selection (Heide and Miner, 1992). However, while it is difficult to rule out the influence of these factors in our study, the abundance of communal signals in species that are either socially monogamous or group-territorial with low intra-group relatedness suggests a prominent role for the simpler theory of interdependence (Roberts, 2005). Under this view, individuals cooperate over signal production because of the direct fitness benefits of collaboration–that is, individuals are more likely to maintain positions in pairs or groups and to defend adequate resources to reproduce if they coordinate signaling as a team.

Challenges and Opportunities

Synthesizing current information on territorial behavior, social stability and communal signaling across the world's birds is difficult given the lack of published studies for most species. Nonetheless, by incorporating multiple strands of evidence, including direct observations, experiments, and expert knowledge, we believe our dataset provides the most robust and comprehensive estimate of current knowledge to date. Our classification of species into broad categories means that, although some error is unavoidable, the majority of lineages are very likely assigned correctly. Moreover, the sample size is large enough to absorb considerable noise and we suspect that the key patterns reported are so striking that future adjustments will have little influence on the main results. This conclusion is strongly supported by sensitivity analyses showing that our results are robust to variation in data quality.

In effect, we have followed the model adopted by many prominent studies of climate change, or the IUCN Red List categories of conservation status (IUCN, 2001). The Red List uses arbitrary thresholds to assign species to threat categories in all but the most data-poor scenarios, often on the basis of expert opinion. Despite the drawbacks and early criticisms of this approach, it has been shown to be largely accurate, and has proved to be an extremely valuable tool for a prolific field of research (Rodrigues et al., 2006). While further revisions and corrections are inevitable, we hope the classifications presented here provide a similar template for further study, both to refine the dataset and to underpin broad-scale tests of evolutionary theory, in line with previously published datasets of similar scope (Cockburn, 2006; Jetz and Rubenstein, 2011; Wilman et al., 2014).

CONCLUSIONS

Based on our global survey, we estimate that communal signaling occurs in at least 1830 (∼18 %) bird species, and is thus far more widespread than often assumed. Our analyses confirm that the occurrence of this behavior across the world's birds is correlated with a suite of environmental variables, including climatic variability and latitude, as well as migratory behavior and cooperative breeding. However, all these associations appear to be secondary because they are largely or entirely explained by a combination of long-term territory ownership and social bonds. We propose that the value and defendability of ecological resources, and the fluctuation of their value and defendability over time, are key factors driving the evolution of communal signaling. Competition for defendable resources may promote the formation of stable coalitions, theoretically increasing the degree of interdependence and collaboration among individuals. Given that communal signals are by definition produced by females as well as males, it seems likely that similar processes also play a prominent role in maintaining songs in females. However, further studies are required focusing more explicitly on patterns of female song in birds, including species where females sing independently from males.

AUTHOR CONTRIBUTIONS

JT and NS conceived, designed, and coordinated the study, and wrote the manuscript. JT and AC compiled and curated data. CS, AM, and SN designed and carried out statistical analyses.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: http://journal.frontiersin.org/article/10.3389/fevo.2016.00074

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Typical Males and Unconventional Females: Songs and Singing Behaviors of a Tropical, Duetting Oriole in the Breeding and Non-Breeding Season

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Recent research emphasizes that female song is evolutionarily important, yet there are still few species for which we have quantified the similarities and differences between male and female song. Comparing song rates and the structure of female and male song is an important first step to forming hypotheses about functional and evolutionary differences that may exist between females and males, especially in year-round territorial species that may use their songs for breeding and non-breeding activities. We compared female and male singing rates and song structure in a tropical New World oriole, the Venezuelan troupial (*Icterus icterus*) during both the breeding and non-breeding season and between the dawn and day. Males sang solos at particularly high rates during the breeding season before dawn. Females, however, sang at consistent rates year-round, primarily during the day. Females answered 75% of male day songs, producing duets, whereas males answered only 42% of female songs. Duets were common year-round, but occurred more often during the non-breeding season. Structurally, female songs were higher pitched and shorter than male songs. We detected no sex differences in the number or order of syllables, however, interestingly, answers were shorter than duet initiations and solos, and, during the breeding season, songs that initiated duets were characterized by higher syllable diversity than were answers or solos. The fact that males sing more during the breeding season supports the classical hypothesis that male song is a sexually selected trait. However, our findings that females sing solos and answer the majority of male songs to create duets year-round suggests that female song may have evolved to serve multiple functions not exclusively tied to breeding.

Keywords: female song, duet, troupial, *Icterus*, behavior, selection

INTRODUCTION

Most bird song research has been conducted on temperate male songbirds and supports the hypothesis that male song is shaped by sexual selection to attract mates and defend territories (Beecher and Brenowitz, 2005; Catchpole and Slater, 2008). Conversely, very little information exists on the function or even the structure of song in female songbirds (Langmore, 1998; Riebel et al., 2005). However, recent research provides evidence that female song is common and was likely ancestral in songbirds (Odom et al., 2014). Thus, to comprehensively investigate the evolution of song in songbirds, we need to study both male and female song. Comparing the structure and timing of female and male songs is a first step in understanding the selection pressures that may be acting on the songs of each sex (Hall, 2004; Dahlin and Benedict, 2014).

Female song is particularly common in the tropics and unlike in temperate regions tropical songbirds may sing throughout the breeding and non-breeding seasons (Morton, 1996; Stutchbury and Morton, 2001). While temperate male songbirds use song to gain access to mates, female animals may use elaborate traits to compete for resources that increase their survival and fecundity (Emlen and Oring, 1977; West-Eberhard, 1983). Elaborate female traits, therefore, may evolve through broader selection pressures, such as social or natural selection to compete for and maintain multi-purpose territories or non-breeding resources (Lebas, 2006; Tobias et al., 2012; Clutton-Brock and Huchard, 2013). We propose, one way to assess whether a trait has been shaped by sexual selection is to examine whether the trait is used exclusively during the breeding season. Traits that are used well outside of the breeding season may have evolved in response to social or natural selection rather than solely sexual selection (Tobias et al., 2012).

Most studies that have directly compared male and female songs have compared structural variation (Logue and Gammon, 2004; Mennill et al., 2005; Rogers, 2005; Brunton and Li, 2006; Hall, 2006; Molles et al., 2006; Dowling and Webster, 2013; Hahn et al., 2013; exceptions: Price et al., 2008; Topp and Mennill, 2008; Illes and Yunes-Jimenez, 2009). Comparing structure of male and female song has revealed interesting trends in the overall complexity or repertoire sizes of male vs. female song. This has been used to assess the relative role of sexual selection in shaping elaboration of male vs. female traits (Mennill et al., 2005; Brunton and Li, 2006). Similarly, structural similarity and sex-specific song features have been useful for evaluating sex-role similarity and individual motivation, especially in duetting species (Logue and Gammon, 2004; Rogers, 2005; Hall, 2006).

In contrast to song structure, singing behavior and song rates may be especially informative when considering the breadth of selection pressures that impact tropical species that live in the same location year-round (Price et al., 2008; Illes and Yunes-Jimenez, 2009). Outside of the breeding season, singing is more likely to mediate competition for resources or social interactions not related to mating. Comparing male and female song rates between the breeding and non-breeding seasons is valuable for determining the relative importance of male and female songs in regulating breeding vs. non-breeding activities (Tobias et al., 2012). Two studies report female song rates that exceed male song rates (Price et al., 2008; Illes and Yunes-Jimenez, 2009). However, no studies have examined how male and female song rates vary between the breeding and non-breeding season. Here, we quantify the song rates, as well as song structure of male and female Venezuelan troupials (*Icterus icterus*, hereafter "troupials") in both the breeding and non-breeding seasons to provide insight about the function and selection pressures acting on male and female song.

Troupials, a New World oriole, are a sexually monochromatic, socially monogamous, tropical-breeding songbird (Jaramillo and Burke, 1999). Mated pairs maintain territories together year-round and both males and females participate in territory defense (Odom unpublished data). Territories are predominantly non-overlapping and nest sites are solitary (Odom unpublished data; Jaramillo and Burke, 1999). No prior studies have been conducted on banded populations of troupials and there is no formal description of their vocalizations. Previous accounts describe troupial songs as comprising 2–3 repeated, alternating low and high "whistles" (pure tones) with distinct space between notes (Jaramillo and Burke, 1999). Both males and females sing solos and mated pairs often overlap each other's songs to form duets (Odom, pers. obs.). Existing accounts also mention high rates of singing at dawn, but suggest that males are the primary singers (although others have not had banded populations; Jaramillo and Burke, 1999). Sex-specific seasonal variation in dawn singing has not been documented in tropical songbirds where female song is common. Therefore, we were also interested to quantitatively compare troupial songs and song rates between the dawn and day. Troupials are native to NE Colombia, most of Venezuela, Aruba, Curacao, and Isla Margarita, but are considered introduced to islands throughout the Caribbean including Puerto Rico, St. Thomas, the U.S. Virgin Islands, and Bonaire (Raffaele et al., 1989; Jaramillo and Burke, 1999).

We offer the first comprehensive description of variation in male and female troupial songs (**Figure 1**). Our paper has two goals: (1) quantify the singing behavior and song structure variation in male and female troupials, and (2) determine whether male and female singing behavior and song structure varies as a function of season (breeding vs. non-breeding) or time of day (dawn vs. day). We discuss our results in the context of existing information on the function and selection pressures acting on male and female songs and duets.

Methods

We collected data over three field seasons in the dry forests of Cabo Rojo National Wildlife Refuge in southwestern Puerto Rico (17°590 N, 67°100 W), constituting two breeding seasons and one non-breeding season. We recorded Troupials from 10 May to 30 June 2013, 22 April to 01 July 2014, and 09 November to 18 December 2014. Late April to early May is the beginning of the wet season in Puerto Rico and the start of the breeding season for troupials in the dry forests of the island. October to April is the dry season, which is the non-breeding season for troupials in our study area. Our non-breeding season research was conducted in the first half of the non-breeding season, and thereby avoiding any confounding effects of pre-breeding activities.

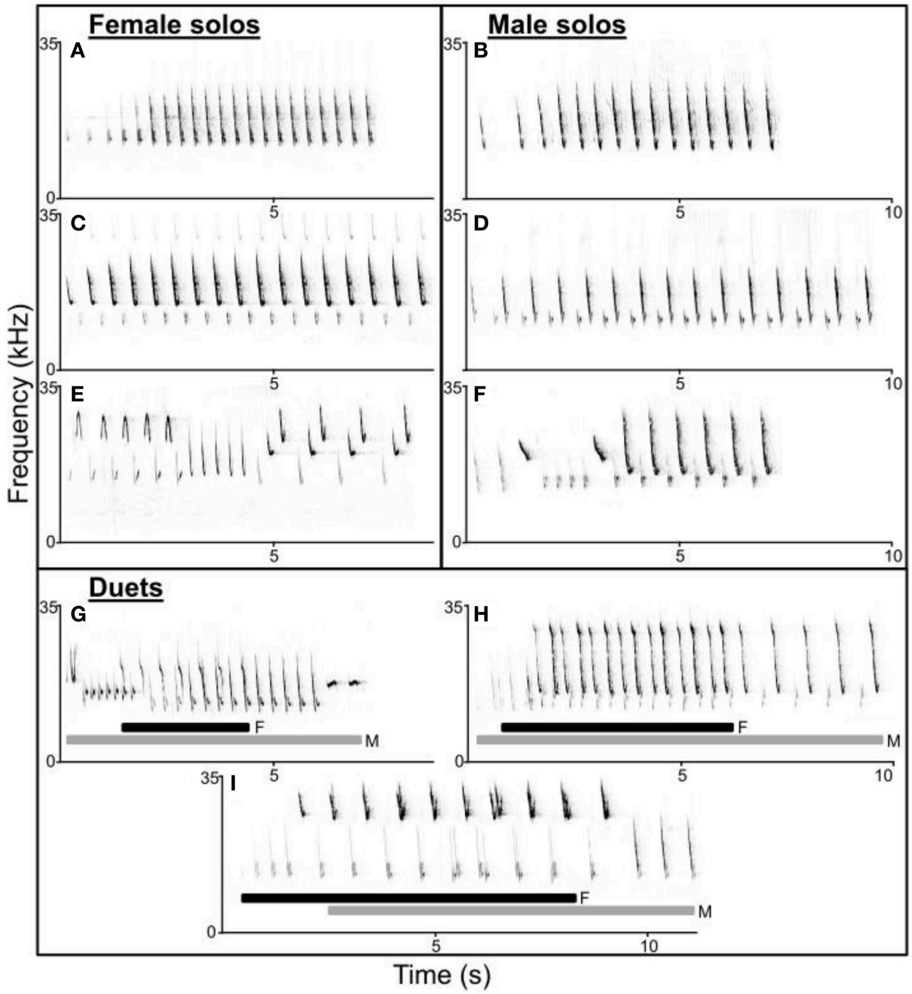

FIGURE 1 | Spectrograms of troupial (*Icterus icterus*) female (A,C,E) and male (B,D,F) solos and duets (G–I). Female and male songs have similar structure **(A–D)** and range from 1 to 3 alternating syllables most often used in repeating motifs. Both female and male songs can have simple **(A,B)** or complex **(E,F)** syllable diversity and order. Duets vary structurally including both male-initiated (**G,H**; female part of duet underlined by black bars) and female-initiated (**I**; male part underlined by gray bars) duets. In addition, both songs and syllables within duets may overlap to varying extents and troupials may use different **(G)** or similar **(H,I)** song types as their mate within duets.

For this study, we focused only on mated, territorial pairs of troupials. Over the three field seasons, we regularly recorded 16 pairs of troupials. Turnover in mates and territories resulted in a final sample size of 19 males and 18 females (see Supplementary Table 1 for a full list of sample sizes and number of songs included in each analysis). All troupials were banded with individual and sex-specific celluloid color bands and a USGS aluminum band. Males were fitted with an aluminum band over a light blue band on their left leg whereas females received a light pink over aluminum band on their right leg. Troupials are sexually monochromatic to human observers, so the colored leg bands allowed us to identify sexes in the field. Sex was tentatively assigned in the field by wing length measurements. Males on average have a wing length of 118.9 mm (±3.3 mm SD) while female wing lengths average 110.9 mm (±2.4 mm SD). In the field, sex identification was aided by the presence of a brood-patch or cloacal protuberance during banding, and breeding activity afterward (e.g., female nest building and incubation). We confirmed sex of our mated pairs using molecular sexing in the lab (Griffiths et al., 1998). In the summer and fall 2014, each male and female of our mated pairs were fitted with a very high frequency (VHF) radio tag to further aid in quick identification and location of individuals (radio transmitter models Pip Ag386 and Ag393 manufactured by Biotrack). We recorded birds with a Marantz PMD 661 recorder and Sennheiser ME67 shotgun microphone with K6 power module. All field methods and protocols were approved by the University of Maryland, Baltimore County IACUC committee (approval number KO010531215) and we had all necessary federal and state permitting (Federal bird banding permit number 23205 and Puerto Rico Department of Natural Resources scientific permit number R-VS-PVS15-SJ-00423-09102014).

We recorded birds during standardized 1-h observation sessions. We conducted three sessions within the first 4 h after sunrise each morning (allowing time between sessions to locate and identify pair members before the start of each observation). We cycled through each pair of troupials before re-observing a pair so that all pairs were regularly observed at least once per week. Each pair was also observed an equal number of times each hour of the morning relative to sunrise. During observations, we recorded and noted male solos, female solos, and duets that occurred within each observation session, as well as which sex sang first or second in the duet when we saw who sang which part of the duet. We defined duets by the presence of temporally overlapping song. We also noted when one or both pair members were not present and adjusted song rates to reflect the proportion of the time birds were present. We did not include songs in the analysis that were given when we did not know the location of at least one pair member or when we were uncertain which bird vocalized. All observations were conducted by two observers so that each observer could focus on watching one member of the pair.

All pairs were recorded at least once at dawn during both the breeding and non-breeding seasons of 2014 (the two seasons we had radio tags). We began dawn recordings approximately 45 min before sunrise and continued recording until the sun was visible on the horizon or the pair left the area (whichever happened first). We used radio tracking to identify and verify the location of each pair member before we began recording. We tracked individuals to within 20–30 m of roosting locations to be confident in each bird's location. We only included recordings in which pair members were far enough away from one another that their radio signals and, therefore, their vocalizations could be separated spatially and the expected locations of individuals based on radio tracking matched the location of vocalizing troupials.

We measured several components of troupial songs, including song rate, frequency, duration, and song syllable composition. Songs for fine-structural measurements and composition were extracted from the full dawn and day time recordings, as well as a few recordings made prior to playback experiments. For frequency measurements, we only included recordings with a strong signal, low noise, and no loud sounds obstructing the song. For temporal measurements and composition, we used only songs for which we could clearly see the beginning and end of the song, as well as the component syllables. There were some songs that we measured for composition but not temporal measurements because syllables could be identified but not measured, or vice versa.

Song Rate

Song rate was calculated for male solos, female solos, and duets within each recording. Daytime song rates were calculated by dividing the number of each vocalization type by the time that one or both pair members were present during an observation session. For dawn songs, we divided the overall number of each vocalization type by the duration of the recording (equivalent to the time we spent in close radio contact with a pair). Ideally, this overall comparison of solo and duet rates would have included the component male and female initiation and answer rates, allowing us to fully reconstruct the individual-level behaviors of males and females. However, we only observed which individual initiated or answered duets in 48% of duets (largely because troupials often fly or are engaged in territorial interactions right before duetting). Therefore, we used the percent of female and male day time solo and duet rates from the above analysis and known duet initiation rates to calculate the percent of male and female total initiations (solos plus songs used to initiate duets) and answers (songs used to respond to a mate's song to form a duet). In other words, we calculated overall initiation and answer rates from the component percentages of solo and duet initiation rates (see **Table 2**). We only used 2014 data for song rate analyses because we only had dawn singing rates for 2014. We only used daytime singing rates for initiation rate calculations because very few duets or female solos are produced at dawn.

Structure

All fine-structural measurements were completed in RavenPro v1.4 (Bioacoustics Research Program, 2011).

Frequency Measurements

We used RavenPro's automated measurements to provide standardized measures of frequency based on the energy of the signal rather than the spectrogram itself, including 95% frequency (upper frequency), 5% frequency (lower frequency), and 90% frequency bandwidth (Bioacoustics Research Program, 2011; Zollinger et al., 2012). We measured these parameters for male and female solo songs; substantial overlap of syllables within duets prevented accurate frequency measurements for duet initiations and answers. Frequency measurements were conducted using the Hann window function and transform size 1024 points for an effective frequency resolution of 5.8 Hz. We also measured peak frequency (the frequency at peak amplitude), however, peak frequency was highly correlated with all other frequency measures, so we only include analyses of the previous three frequency measures.

Temporal Measurements

We measured total duration of male and female solo songs as well as the component male and female songs from within duets. For temporal measurements, we used a transform size of 256 points and we viewed only 4 s of each song at a 100% window width for an effective time resolution of 0.003 s. We used the end time subtracted from the begin time of a selection encompassing the entire song to calculate total duration of each song. We estimated the beginning and end time of each song on the spectrogram, but compared the spectrogram to the waveform while making our measurements to increase our ability to precisely determine where the first syllable began and last syllable ended.

Syllable Composition

Male and female solo songs, duet initiations, and answers were measured for composition analyses. Syllables were separated into discrete syllable types based on overall shape and frequency range. Each syllable type was assigned a distinct letter code and a graph network-based approach was used, wherein nodes represent syllable type letter codes, and edges represent transitions between syllables (Sasahara et al., 2012). Four aspects of song composition were quantified: (1) number of syllables per song, (2) syllable diversity (number of syllable types per syllable), (3) number of transitions between syllable types, and (4) the average minimum path length between all pairs of syllable types in the graph network. We did not convert transitions to a rate because doing so resulted in non-normally distributed principal components. Average minimum path length (hereafter "path length") is a graph network metric that measures the average minimum distance between nodes (i.e., pairs of syllables). Path length quantifies an aspect of syntactical orderliness, with longer paths indicating greater order (Sasahara et al., 2012). Average minimum path length was calculated in R using the igraph package (Csardi and Nepusz, 2006; R Core Team, 2015). We used principal components analysis (PCA) to reduce the number of variables and account for correlation among variables. We did not employ rotation because the unrotated solution was satisfactory, and rotation can complicate interpretation of components (Jolliffe, 2002). Bartlett's test of sphericity indicated that data were suitable for PCA ($\chi^2 = 1852.18$, $df = 6$, $p < 0.0001$). A scree plot was used to determine the number of principal components for analysis.

Statistical Analysis

All statistical analyses were performed in R (R Core Team, 2015), except PCA of the composition data, which was conducted in InfoStat v 2012 (Di-Rienzo et al., 2012). Separate analyses were conducted for song rate, frequency, duration, and syllable composition. Song rates were analyzed using non-parametric Kruskal-Wallis analyses. We used non-parametric analyses for song rate because the data were continuous and zero-inflated. We conducted separate, individual tests to compare song rate to the fixed effects of song type (male solo, female solo, or duet), time of day, and season and their two-way interactions. To control for multiple recordings per bird, we averaged song rate by individual and pooled the averages according to song type, time of day, and season for each analysis. We assessed variation in answer rates attributable to sex and season with a generalized linear mixed model (GLMM) of answer rates with fixed effects sex, season, and their interaction, and random effects individual and year using the "lme4" package in R (Bates et al., 2015).

For frequency, duration, and syllable composition analyses we built linear mixed models (LMM) using the "lme4" package in R (Bates et al., 2015). For these analyses, we included sex and season as fixed effects and individual and year as random effects. Frequency analyses only included songs produced as solos, whereas duration and composition analyses included male and female songs both from solo songs as well as the component male and female songs within duets. Therefore, duration and composition analyses included an additional fixed effect of role, which accounted for whether a song was a solo, a duet initiation (the first song sung in a duet), or an answer (the second bird to sing in a duet). Time of day was included as a single fixed effect in a separate LMM than sex, season, and role because we only had enough measureable dawn songs for males during the breeding season. Female songs were too infrequent at dawn to have a large enough sample size for statistical analysis. Therefore analyses with sex, season, and role were restricted to songs sung during the day, whereas analyses including time of day included only males in the breeding season. Analyses with sex, season, and role also included the two-way interaction terms of sex*season, sex*role, and season*role. We dropped interaction terms that did not contribute to a significant portion of the variation in the full model, assessed by backward selection, resulting in a reduced model. To assess significance of main fixed effects and any remaining interactions, we pairwise compared the reduced model to drop-one models containing all remaining fixed effects except the one being assessed. We compared each drop-one model to the full model using an ANOVA F-statistic to produce p-values. We accepted an alpha-level of $p = 0.05$ as statistically significant for all analyses.

RESULTS

Song Rate

Song rate varied significantly depending on song type (male solo, female solo, or duet) as well as with the interaction of song type by season and song type by time of day (**Table 1**; **Figure 2**). There was no effect of season or time of day individually or the interaction of season and time of day (**Table 1**). These differences in song rate were driven primarily by disproportionately high rates of male solos: males sing significantly more solos than do females and most male songs are solos rather than duets (**Table 1**). However, variation in the rates of each song type are driven primarily by interactions with season and time of day, as follows (**Table 1**). Male soloing is prominent at dawn and during the breeding season. Conversely, females sing very little at dawn, but sing more solos than males in both the breeding and non-breeding season (29% of all day songs are female solos vs. only 12% are male solos; **Tables 1**, **2**; **Figure 2**). However, during the day, most often male and female songs are answered to produce duets (**Tables 1**, **2**; **Figure 2**). Duets were produced more often during the non-breeding vs. the breeding season (**Table 1**; **Figure 2**).

When we considered the individual-level behaviors of male and female initiation and answer rates, males and females initiate approximately the same number of songs during the day

TABLE 1 | Song rate analyzed for song type (male solo, female solo, or duet), season (breeding or non-breeding), time of day (dawn or day) and their interactions using a Kruskal-Wallis non-parametric analysis of individual averages.

Data	Response variable	Explanatory variable	*df*	X^2	*p*-value
All songs	Songs per hour	Song type	2	12.75	**0.002**
		Season	1	0.01	0.928
		Time of day	1	0.27	0.603
		Season * Song type	5	48.17	**<0.001**
		Time of day * Song type	5	27.68	**<0.001**
		Season * Time of day	3	6.23	0.101

Values in bold are statistically significantly different based on a p-value of 0.05.

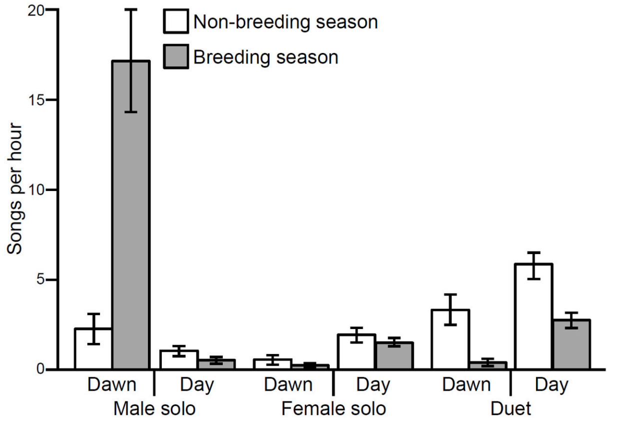

FIGURE 2 | Song rates (per hour) for troupial male solos, female solos, and duets vary significantly from one another ($X^2 = 12.75$, *df* = 2, $p < 0.01$) especially between the breeding and non-breeding season ($X^2 = 27.68$, *df* = 5, $p < 0.01$) and dawn vs. day ($X^2 = 48.17$, *df* = 5, $p < 0.01$). Bars represent means with standard error bars.

TABLE 2 | Sex-specific rates of solo singing, initiation, and duet answers show that males and females initiate about the same number of songs but females answer male songs to form duets at much higher rates than males answer female songs.

Sex	Solos (out of all songs) %	Duet initiations (out of all songs) %	Total initiations (solos + duet initiations) %	Answers
Male	12	37	49	42
Female	29	21	51	75

(including solos and duet initiations; **Table 2**). Females answer 75% of male songs (**Table 2**). Males, however, answer only 42% of female songs (**Table 2**). These values account for the lower rates of male solo singing during the day: females answer a majority of male songs, turning them into duets. Females therefore answer a significantly larger portion of duets (GLMM: sex: $X^2 = 9.14$, *df* = 1, $p < 0.01$). Answering rates do not vary with season (GLMM: season: $X^2 = 0.01$, *df* = 1, $p = 0.92$).

Structure

Troupial solos and duets varied considerably in structure and composition (**Figure 1**). In our population, most male and female songs consisted of 1–3 alternating syllables used in repeated motifs (**Figure 1**). However, while we noticed that individual troupials appear to have repertoires of a few song types (approximately 2–4 song types each for both males and females), song types are not entirely stereotyped. Both male and female troupials may substitute notes from one song with similarly structured notes from another song, or alternate among motifs after variable numbers of phrase repetitions, creating more complex song types (**Figures 1E,F**). The timing and extent of overlap and the song types used in duets also varies widely (**Figures 1G–I**). While simple song types were shared by many individuals in the population, complex song types appeared to be shared among fewer individuals. Although, syllables used to create most song types were widely shared within the population.

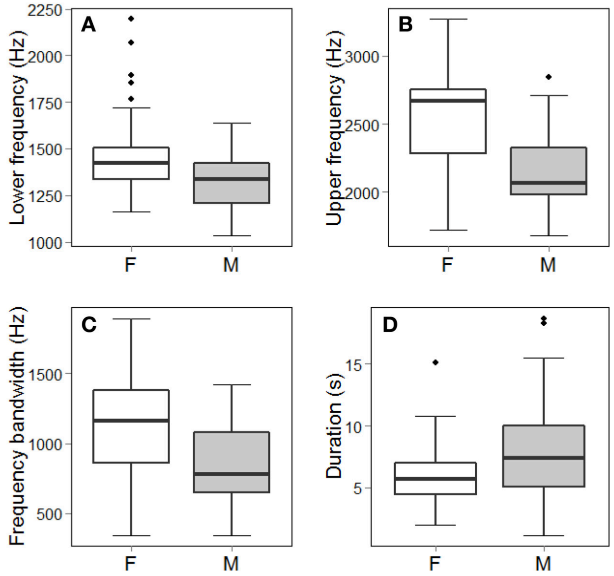

FIGURE 3 | Frequency (A–C) and temporal (D) measurements that were statistically significantly different between female (F) and male (M) song ($p < 0.01$; see Table 4). Note, however, the broad range overlap for all four parameters. Box and whisker plots show the entire distribution of the data, including median, 1st and 3rd quartiles, all data within 1.5 times the Inter Quartile Range beyond the 1st and 3rd quartiles, and outliers.

Frequency Measurements

Female songs were characterized by significantly higher upper and lower frequencies and larger frequency bandwidths than were male songs (**Table 4**; **Figure 3**). There was, however, substantial frequency overlap between male and female song (**Figure 3**). Frequency measurements of male and female songs did not differ between seasons (**Table 4**). Upper frequencies and frequency bandwidth did not vary between male dawn and day songs (**Table 4**). The lower frequencies of male dawn songs, however, were marginally significantly higher than those of male day songs (**Table 4**). Interaction terms did not contribute to a significant amount of variation (Lower frequency Sex*Season: $X^2 = 0.004$, *df* = 1, $p = 0.94$; Upper frequency Sex*Season: $X^2 = 1.39$, *df* = 1, $p = 0.24$; Frequency bandwidth Sex*Season: $X^2 = 1.31$, *df* = 1, $p = 0.25$).

Temporal Measurements

For temporal measurements, there were significant effects of sex, season, role, and time of day (**Table 4**). Males produced significantly longer songs than females (**Figure 3**; **Table 4**). However, the range of female song durations falls entirely within the range of male song durations (**Figure 3**). Songs sung during the breeding season are shorter than songs sung in the non-breeding season (**Table 4**). Songs used as answers are significantly shorter than duet initiations (**Table 4**), but neither duet initiations nor answers were significantly different from solo songs (Tukey's *post-hoc* test of LS means: answerer–initiator: t-ratio $= 2.85$, $df = 461$, $p = 0.01$; solo–initiator: t-ratio $= 0.95$, $df = 458$, $p = 0.61$; solo–answerer: t-ratio $= -2.11$, $df = 467$, $p = 0.09$). Lastly, male dawn solos were significantly longer than male daytime solos (**Table 4**). Interaction terms did not contribute to a significant amount of variation (Sex*Season: $X^2 = 3.05$, $df = 1$, $p = 0.08$; Sex*Role: $X^2 = 1.94$, $df = 2$, $p = 0.38$; Season*Role: $X^2 = 1.52$, $df = 2$, $p = 0.47$).

Syllable Composition

Scree plot analysis indicated that the first two principle components were appropriate for analysis (eigenvalues = 2.04 and 1.57). Together, they explain 90% of the variation (PC1 = 51% and PC2 = 39%) in the composition variables. High values of PC1 characterize songs with many syllables and many transitions between syllable types (**Table 3**). High values of PC2 characterize songs with many syllable types arranged in an orderly syntax.

The composition of songs sung during the day (both within duets and as solos) did not differ by sex (**Table 4**). The significant effect of season on PC1 indicates that non-breeding season songs were longer and contained more transitions than did breeding season songs. Answers were characterized by fewer syllables and transitions than either solos or initiations, but solos and initiations were statistically indistinguishable (Tukey's *post-hoc* test of LS means: answer vs. initiation: t-ratio $= -3.90$, $df = 614$, $p = 0.0003$; answer vs. solo: t-ratio $= -4.06$, $df = 613$, $p = 0.0002$; initiation vs. solo: t-ratio $= 0.23$, $df = 606$, $p = 0.97$).

There was a significant role-by-season interaction in the PC2 model. Examination of the interaction plot revealed that between-season changes in initiations were the primary driver of this interaction (**Figure 4**). Specifically, initiations had higher syllable type diversity and path lengths in the breeding season than they did in the non-breeding season (initiation breeding vs. initiation non-breeding: t-ratio $= -3.58$, $df = 253$, $p = 0.0055$). The only statistically significant role-by-season comparison that did not involve breeding season initiations indicated that breeding season answers had lower values of PC2 than did breeding season solos (t-ratio $= -3.01$, $df = 601$, $p = 0.033$).

We did not detect significant compositional differences between male songs that were sung before dawn, vs. those sung during the day (**Table 4**). The residuals of LMMs for all structural analyses were approximately normal and homoskedastic.

DISCUSSION

Troupials have notable sex differences in their singing behavior. Males sing most during the breeding season at dawn, whereas females sing consistently year-round during the day. Similar to female songs, duets are produced year-round, but duets occur at higher rates during the non-breeding season. Females appear to play a large role in shaping daytime vocal behavior: females and males initiate similar numbers of day songs, but females answer a majority of male songs to create duets. Male and female troupial songs exhibit some differences in frequency and duration, but have similar syllable composition. These results suggest male and female song structure is under similar selection pressures, but that the selection pressures influencing male and female singing behavior varies. While key aspects of male singing behavior seem to be under classical breeding season pressures, female singing behavior may be influenced by a range of selection pressures that likely includes sexual as well as broader social or natural selection pressures.

TABLE 3 | Principle Component Analysis (PCA) loading values for troupial song composition data.

Variable	PC1	PC2
Number of syllables	0.65	−0.17
Syllable diversity	−0.21	0.74
Number of transitions	0.64	0.05
Path length	0.35	0.65

Song and Duet Rates and Selection Pressures

The year-round occurrence of duets but increased rate in the non-breeding season suggests that song answering likely functions in resource defense (Hall, 2004). Troupials eat fruits and insects, resources that may be particularly limited during the dry, non-breeding season (Skutch, 1996; Jaramillo and Burke, 1999). To defend an area suitable to encompass sufficient amounts of these resources may require increased territory defense in the non-breeding season. In addition, the non-breeding season may be a time of year when hatch-year birds are dispersing and attempting to establish territories. Increased territory defense may be required at this time of year to deter dispersing birds from foraging or settling within inhabited territories.

We were surprised to find that female answer rates exceeded male answer rates. Sex differences in answering rates could evolve as a consequence of sex differences in the costs

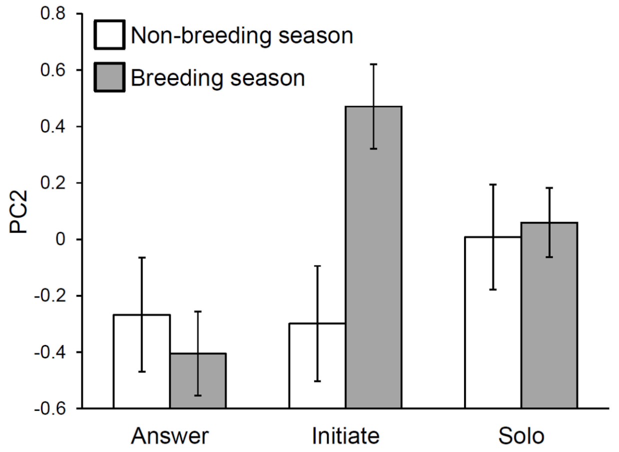

FIGURE 4 | An interaction between singing role and season explains variation in troupial song composition. Composition PC2 measures syllable diversity and average minimum path length between syllable types. Seasonal differences in initiating songs are the primary driver of the interaction effect. Bars represent least squares means with standard error bars.

TABLE 4 | Linear mixed effect models indicate the relative effects of sex (male or female), season (breeding or non-breeding), role (solo, duet initiation, or answer) and time of day (dawn or day) on structural frequency, temporal, and composition measurements of troupial songs.

Measurement	Data	Response variable	Explanatory variable	Value	SE	*df*	X^2	*p*-value
Frequency	Male and female day solos	Lower frequency	**Sex: male**	−107.55	59.41	1	7.01	**<0.01**
			Season: breeding	−50.48	31.73	1	3.16	0.08
		Upper frequency	**Sex: male**	−486.21	112.74	1	20.87	**<0.01**
			Season: breeding	−27.06	58.78	1	0.03	0.87
		Bandwidth	**Sex: male**	−381.45	118.15	1	11.54	**<0.01**
			Season: breeding	24.60	60.86	1	1.21	0.27
	Male dawn and day solos	Lower frequency	**Time of day: day**	−60.51	30.06	1	3.87	**0.05**
		Upper frequency	Time of day: day	24.57	76.11	1	0.10	0.75
		Bandwidth	Time of day: day	80.81	72.48	1	1.20	0.27
Temporal	All day songs	Duration	**Sex: male**	2.42	0.67	1	10.45	**<0.01**
			Season: breeding	−1.27	0.54	1	21.90	<0.01
			Role:			2	8.72	**0.01**
			Soloist	−0.18	0.54			
			Answerer	−1.24	0.55			
	Male dawn and day solos	Duration	**Time of day: day**	−2.58	0.6	1	17.91	**<0.01**
Composition	All day songs	PC1	Sex: male	0.30	0.23	1	1.84	0.18
			Season: breeding	−0.31	0.12	1	6.54	**0.01**
			Role:			2	20.36	**<0.01**
			Soloist	0.55	0.14			
			Initiator	0.58	0.15			
		PC2	Sex: male	0.11	0.19	1	0.35	0.55
			Season: breeding	−0.14	0.20	3	16.46	**<0.01**
			Role:			4	27.58	**<0.01**
			Soloist	0.27	0.20			
			Initiator	−0.03	0.21			
			Season * Role:			2	13.28	**<0.01**
			Breeding—Initiator	0.91	0.27			
			Breeding—Soloist	0.19	0.25			
	Male dawn and day solos	PC1	Time of day	0.16	0.24	1	0.008	0.93
		PC2	Time of day	−0.16	0.18	1	0.75	0.39

*See text and **Figure 4** for post-hoc pairwise comparisons and details of the interaction effect, respectively. Values in bold are statistically significantly different based on a p-value of 0.05.*

and benefits of song answering (Hall, 2004). Temporally overlapping answers by one sex have been cited as evidence of mate guarding by the overlapping sex (Sonnenschein and Reyer, 1983; Tobias and Seddon, 2009; Dowling and Webster, 2015). In troupials, there is no obvious reason for females to guard mates, especially during the non-breeding season, and indeed, both sexes of troupials overlap the mate when answering. Females, however, could overlap male songs in the non-breeding season to prevent usurpation of mates by advertising that their male is taken at a time when many young females may be available and establishing new territories. This presents an interesting scenario where female answering could prevent mate loss outside of the breeding season in a way that ties into pair-bond maintenance for the breeding season. However, it is important to note that our analyses were conducted on already mated pairs of troupials, so the abundance, timing, and selection pressures on singing behavior might vary among unmated individuals. Moreover, pre-breeding activities represent a time when physiological changes pertaining to breeding activity are occurring, so would be a particularly interesting time to examine sexually selected pressures on singing behaviors.

Sex Differences in Song Structure

Female troupial songs are higher pitched and shorter than male songs. Females also have a larger overall frequency bandwidth compared to males, caused by the fact that the high frequencies within female songs are disproportionately higher than their lower frequencies (**Figure 3**). Female songs are also shorter than male songs, yet, we did not find any differences in number of syllables or syllable composition between male and female songs, suggesting that female songs may just be faster paced. Indeed, visually, some female songs appear to have slightly shorter inter-note intervals than male songs. This is interesting, in the context of selection pressures and performance trade-offs that are known to exist between frequency bandwidth and syllable rate for certain temperate male songbirds (Podos, 1997; Podos and Nowicki, 2004; Byers et al., 2010). However, information is needed on whether females are actually singing more rapid songs than males.

The frequency and temporal differences we detected between male and female troupial songs were subtle; they would not allow for sex identification with high confidence (**Figure 3**). Moreover, we did not detect any differences in note composition between the sexes. Overall, troupial songs are remarkable for their relative lack of sexual dimorphism. Some species, including some monochromatic species, exhibit distinctive sex-specific songs or structural features (Mennill et al., 2005; Rogers, 2005; Logue et al., 2007). However, in other species, males and females have similar overall structure, but differ in a few, continuous parameters, such as frequency or number of syllables (Mennill et al., 2005; Brunton and Li, 2006; Illes and Yunes-Jimenez, 2009). Some of these species have fairly substantial differences in pitch or syllable number that even allows researchers to distinguish among males and females (Mennill et al., 2005). In still other species, males and females exhibit different levels of vocal complexity that may be indicative of selection for more elaborate songs in one sex over the other (Brunton and Li, 2006; Illes and Yunes-Jimenez, 2009). However, we suggest that troupials exhibit less sexually distinct songs than most previously examined species, supported by the substantial overlap of frequency and duration of their songs (**Figure 3**). While it is important to examine the possibility of sex differences, a lack of or reduced sexual variation may also be biologically meaningful or informative, especially in a sexually monochromatic, year-round territorial species that may share sex-roles.

Differences in Song Composition between Initiators and Answers

We observed that song structure depended on whether the song was used as a solo, to initiate a duet, or to answer within a duet. This finding emphasizes the importance of examining individual-level analyses within duets that allow for variation among initiators and answers, in addition to sex-specific variation.

Our finding that songs sung as answers have fewer syllables and less order than solos or duet initiations complements our finding that answers are also shorter than duet initiations. We conclude that birds use short songs to answer their mates. More interestingly, during the breeding season duet initiations were characterized by substantially higher syllable diversity and syntactical structure than either solos or answers. We offer three possible explanations for this finding. First, initiators "know" they are likely to be answered and sing a specific song type. Second, initiators may begin solos and initiations the same (e.g., because they do not "know" if they will be answered), but then change them after they have been answered (Tobias and Seddon, 2009). Third, mates may preferentially answer more complex songs. Testing these alternative hypotheses by examining the fine-scale structure of initiations relative to the timing of answers would shed light on the mechanisms and functions of duet singing in this and other species that duet by overlapping the mates' songs.

Seasonal and Diel Variation

Temporal and composition analyses revealed that non-breeding season songs are longer and contain more transitions than do breeding season songs. Such seasonal variation is consistent with the general trend toward more day time song output in the non-breeding season. This further supports the hypothesis that one function of troupial songs is to mediate competition for scarce resources in the dry season.

Overall, there was little variation in the structure of male songs between dawn and day despite greatly heightened song rates at dawn. Dawn songs were longer than day songs and had higher low frequencies. Male dawn and day songs had similar syllable composition. Since male songs are longer at dawn with little differences in syllable number or order, perhaps males sing slower at dawn. Based on visually comparing male dawn and day songs, this appears to be true for some males, but varies by song type.

CONCLUSIONS

Female vs. male singing behavior differs in ways that have interesting implications for understanding selection pressures acting on females and males. Increased male solo singing rates during the breeding season at dawn are consistent with the classical view of male song as a trait that has evolved under the influence of female choice. The use of both male and female song year-round, both as solos and duets, indicates that male and female song also serve functions that are not tightly tied to mating. The increased rate of duetting in the non-breeding season specifically supports the use of duets for year-round territory defense. The finding that female answer rates exceed male answer rates could be viewed as supporting the hypothesis that answering functions in mate guarding or it could reflect the different ways in which males and females participate in resource defense (Hall, 2004). Male and female songs were surprisingly similar in structure suggesting that sex-roles are fairly similar, as might be predicted in year-round territorial species (Stutchbury and Morton, 2001). Variation in troupial song structure based on whether a bird initiates or answers a duet indicates that troupial song composition may be more flexible than traditionally recognized, especially compared to songbird species that crystalize song early in life.

AUTHOR CONTRIBUTIONS

This research is part of dissertation research of KJO with supervision by advisor KEO and committee member DL at all stages. KJO, KEO, and DL all contributed to the conception and formation of the research ideas. Field work was predominantly carried out by KJO and MM. KJO, MM, JC, DM, NR, and DL all contributed to data collection and processing. Statistical analysis was completed by KJO, DM, and DL. The manuscript was written by KJO, DL, and KEO with contributions and comments by all authors.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: http://journal.frontiersin.org/article/10.3389/fevo.2016.00014

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Female song and vocal interactions with males in a neotropical wren

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Bird song is thought to function primarily in same-sex competition, mate attraction, and reproductive stimulation of a partner. However, these conclusions are based largely on studies of the song of male birds in north-temperate species. We investigate female song in a Neotropical wren, *Thryophilus pleurostictus*, using observations and experiments to test the function of female song. Female banded wrens sang much less often than males, their songs were shorter, and their repertoire of song types was smaller. Females did not seem to sing for same-sex competition for resources or mates: female song rate did not increase in response to simulated intrusion, and females sang in response to less than one-third of playbacks simulating territorial intrusion by either unpaired or paired females. Territorial defense is important for both survival and reproduction in species that occupy all-purpose territories year-round, but female involvement in territorial defense was limited. Females were more likely to approach simulated intruders when their partner approached more closely, and were closer to their partner during playback simulating a pair of intruders, perhaps contributing to defense jointly with their partner. Females did not appear to use song to attract males for mating: only 25% of females sang in response to playback simulating an unpaired male during the nest-building period, and they were less likely to sing shortly before laying when they were more likely to be fertile. Female song in banded wrens seemed to be used primarily for communicating with their breeding partner: female songs overlapped or began within 1 s of a song by their partner more often than expected by chance, and male vocal behavior changed in response to song by their partner. However, the low rate of female song in banded wrens suggests this function does not select for song elaboration, consistent with the view that same-sex competition is the main driver of female song elaboration.

Keywords: female song, female competition, mate attraction, duet, playback experiment

INTRODUCTION

Vocal and visual ornamentation of females was traditionally considered rare, with Darwin (1871) proposing that sexual dimorphism in ornamentation was due to sexual selection acting on males, and that examples of female ornamentation were due to shared inheritance of male characteristics. However, recent work on bird song has shown that song is widespread among female songbirds, and that female song was most likely present in the ancestor of modern songbirds (Odom et al., 2014). Thus, selection acting on females may be as, if not more, important than selection acting on males as a cause for sexually dimorphic bird song. Despite the prevalence of female song, much less is known about all aspects of female than male song (Riebel et al., 2005), including its function in inter- and intra-sexual competition and reproductive stimulation of a partner (primary functions of song in males, Catchpole and Slater, 2008). Since the theoretical framework for understanding the evolution of song was based largely on species where female song was rare, the role of vocal interactions with a breeding partner was generally not considered. However, most work on female song has been conducted in duetting species, where females often coordinate their songs with those of their partners to form joint acoustic displays (reviewed in Hall, 2004, 2009). Less is known about female song in species without conspicuous duets. Similarities and differences with male song, and the relative importance of female song for female–female competition, male attraction, and within-pair communication are all poorly understood.

Across taxa, females with visual or acoustic ornaments most commonly use them to compete for resources like territories and nest sites that are necessary for survival and reproduction (Tobias et al., 2012; Clutton-Brock and Huchard, 2013). Competition for resources is often sex-specific (females compete primarily with other females), but a comparative analysis of birds has shown that the same-sex bias in territorial defense behavior is considerably reduced in duetting species compared to non-duetting species (Logue, 2005). It is likely to be costly for females in size-dimorphic species to defend resources against males, but these costs of defense could be reduced if females engage in competitive behaviors jointly with a partner or group (for example, female lions, *Panthera leo*, roar in chorus to defend territories that are essential to reproduction and survival, McComb et al., 1994; Mosser and Packer, 2009). Thus, females may defend resources against females only or against both males and females (or, in principle, against males only). Furthermore, females may defend resources independently, or jointly with a partner.

Females in a range of taxa sometimes use ornamental traits to compete with other females for mates or to attract mates (Tobias et al., 2012; Clutton-Brock and Huchard, 2013). There are a few examples of female song functioning to attract males or compete with other females for males. Female song attracts males in alpine accentors *Prunella collaris* (Langmore et al., 1996). Female dunnocks *Prunella modularis* in multi-female groups are more likely to sing complex songs (Langmore and Davies, 1997). Females in this species compete for male parental investment and females in multi-female groups have reduced reproductive success (Davies, 1986), but we do not know how reproductive success of females in multi-female groups compares for those that sing vs. those that do not sing. Female eastern whipbirds *Psophodes olivaceus* sing highly synchronized answers to male song that form duets and function to defend their mated position against rival females in a system where offspring survival depends on exclusive access to male care and there is a female-biased sex ratio among unmated birds (Rogers and Mulder, 2004; Rogers et al., 2007).

Female song may also function in communication with a breeding partner to maintain group cohesion or to coordinate or stimulate breeding activity (Langmore, 1998; Hall, 2004, 2009). Species occupying dense habitat may need to maintain contact acoustically if visual contact is not possible (Thorpe, 1963; Mays et al., 2006). Biparental care is common in birds (Cockburn, 2006), and the ability of the sexes to coordinate and cooperate in the provision of care is likely to influence reproductive success (Halkin, 1997; Elie et al., 2010; Mariette and Griffith, 2012). Since calls are also suited to maintaining contact, such functions may not select for complex songs. Although greater complexity in male song is more effective at stimulating nest-building in females (Kroodsma, 1976), it is not known whether female song has a similar effect on males.

In species where both sexes sing, female songs may occur in close temporal association with those of their partner by chance, or they may be coordinated strategically to form duets. Song coordination is obviously strategic when the timing or types of male and female phrases within their synchronized songs are highly coordinated (for example, Mann et al., 2003; Hall, 2006; Logue, 2006). However, when entire songs of males and females occur close together in time without any temporal coordination of phrases within the songs (for example, Mennill and Vehrencamp, 2005; Hall and Peters, 2008), formal testing is necessary to distinguish duetting from chance temporal association. Song-type matching (or duet codes) and song overlapping (or alternating) provide evidence of strategic vocal interactions between partners rather than chance associations between their songs (Logue, 2007b), in much the same way that matching song-types and overlapping or alternating songs indicate when males are directing signals at a particular counter-singing male in a network (Searcy and Beecher, 2009).

The banded wren (*Thryophilus pleurostictus*) is a Neotropical wren in which both sexes sing (Molles and Vehrencamp, 1999). Female song is rare, and the species does not perform the highly coordinated antiphonal duets that characterize some related species (Molles and Vehrencamp, 1999; Mann et al., 2009). However, females sometimes participate in boundary disputes with their mates (Molles and Vehrencamp, 1999) and respond to playback of male song (Hall et al., 2006). Response to playback of female song has never been tested in this species, but male-male counter-singing interactions have been characterized in detail, based on observations and experiments (Molles and Vehrencamp, 2001; Burt and Vehrencamp, 2005; Hall et al., 2006; Illes et al., 2006; Vehrencamp et al., 2007, 2014; De Kort et al., 2009), as have male-female duets in many closely related species (Levin, 1996; Logue and Gammon, 2004; Gill et al., 2005; Marshall-Ball et al., 2006; Mennill and Vehrencamp, 2008). The singing style of banded wrens is similar to their congeners, rufous-and-white wrens (*Thryothorus rufalbus*) and sinaloa wrens (*Thryothorus sinaloa*), where rare female songs are occasionally loosely associated with the songs of their partner (Mann et al., 2009). Rufous-and-white wren duets are used in both within- and extra-pair communication, allowing partners to locate one another in their dense habitat and to jointly defend their territory (Mennill and Vehrencamp, 2008). However, the function of female song in banded wrens is unknown, and both the contexts in which females sing and the nature and significance of any vocal interactions between partners are unclear.

We combined observations and experiments to describe and investigate the function of female song and male-female vocal interactions in free-living banded wrens during the breeding season. We used songs recorded during early morning focal observations on pairs of banded wrens to describe the timing and type of female songs and male-female vocal interactions, as well as to test whether male and female songs were associated more often than expected by chance, or varied with context. We used playback experiments conducted during nest-building to test the effect of simulated intrusion on female song and male-female vocal interactions at a time when sexual conflict was likely to be at its peak. We compared responses to "paired intruders" (male and female song played as "duets") and to "solitary intruders" (male and female song played in separate trials). We tested predictions of key hypotheses for the function of female song and answering song that forms duets (summarized in **Table 1**):

(i) *Intra-sexual territorial defense (female song)* – Females approach and sing more when there is a female intruder (duet and female solo playback) than when no female intrudes (male solo playback). Female songs may overlap those of their partner by chance.

(ii) *Joint territorial defense* – Females approach and sing to all intruders, and respond more when there are more intruders (duet playback). Females are more likely to coordinate their response with their partner during duet playback (greater proximity and song answering).

(iii) *Mate attraction (female song)* – Females sing most when they are fertile if attracting males for extra-pair mating. If

Table 1 | Predictions of hypotheses for the function of female song and answering song.

	Nest-building	Partner distant	Solo Female PB	Solo Male PB	Duet PB	Sex-bias in Solo vs. Duet
(i) Intra-sexual territorial defense			↑		↑	F↑/F↑
(ii) Joint territorial defense			↑	↑	↑ (a↑)	=/=
(iii) Attract mate	↑			↑		M↑/–
(iv) Coordinate breeding	↑					
(v) Locate partner		↑				

Contexts in which female song or answering song (a) (song given in response to partner's song to form a duet) are predicted to be most common under different hypotheses. Sex-biased responses to intruders can reflect closer approach to male than female playback (M), or closer approach to female than male playback (F), when male and female playback are presented separately (Solo) or simultaneously (Duet).

attracting a potential partner, females approach and sing most to an unpaired male (during male solo playback).

(iv) *Coordinate or stimulate breeding activity (female song and answering song)* – Females sing, or answer their partner's songs, most at the start of the breeding cycle (early nest-building).

(v) *Locate partner (female song and answering song)* – Females sing, or answer their partner's songs, from a distance.

MATERIALS AND METHODS

STUDY POPULATION AND APPROACH

We studied a color-banded population of banded wrens at Santa Rosa National Park in the Guanacaste Conservation Area in Costa Rica (10.83°N, 85.61°W) that has been the subject of a long-term research project (for details see Molles and Vehrencamp, 1999; Hall et al., 2009). The species is resident year-round and the sexes are somewhat size-dimorphic (mean mass for 86 females = 18.3 g, for 90 males = 20.3 g). Both sexes contribute to parental care: nest-building is primarily (but not exclusively) by males, incubation is exclusively by females, and both sexes provision offspring.

To quantify (i) female song structure, repertoires and sharing, (ii) contexts of female song, and (iii) male–female vocal interactions, we recorded pairs singing naturally during and following the dawn chorus (approximately 5–7 a.m.). We recorded 12 pairs between 28 April and 2 August 2005 for a total of between 3.0 and 8.8 h each (mean = 6.0 h), on 2–6 days (mean = 3.8 days) at different stages of the breeding cycle. Observers attempted to estimate the distance between the male and female whenever a female song was heard.

To further test the function of female song and vocal interactions between partners, we conducted playback experiments to 16 pairs early in the breeding season, from 7 May to 10 June 2005. The playback stimuli comprised (i) female solo songs, (ii) male solo songs, and (iii) "duets" formed by playing male and female song stimuli simultaneously from two speakers. The "duet" treatment thus comprised loosely coordinated male and female songs (temporal coordination described in more detail below) with no precise coordination of phrases within songs, as is typical of the structure of duets in this species (see Results).

FEMALE SONG STRUCTURE, REPERTOIRES, AND SHARING

We recorded songs using a Sennheiser ME67 directional microphone and Marantz PMD 690 digital solid-state recorder, and used the program Syrinx-PC (www.syrinxpc.com—developed by John Burt, University ofWashington, Seattle) to visualize songs and measure song length. We tested for sex differences in song length by comparing means for males and females within 12 pairs, based on all of their songs. Most female songs were classifiable into types similarly to male song types in this species (Molles, 1999; Trillo and Vehrencamp, 2005). We generated plots of cumulative repertoire size vs. number of songs recorded for each of the 12 females to assess whether we could estimate their repertoire sizes reasonably accurately: only five females with 50 or more songs recorded appeared to reach an asymptote. We computed the standard song-type sharing index among the females ($I_f = 2 N_s/(R_1 + R_2)$, where N_s is the number of shared types between two females and the denominator is the sum of their total repertoire sizes) (McGregor and Krebs, 1982). The equivalent index (I_m) was computed among the 12 males for comparison to the females. Because female repertoires were much smaller than male repertoires, we computed the sharing between a female and her mate as the fraction of her song types that were also present in her mate's repertoire. We computed this same shared fraction of song types between each female and the other 11 study males so that we could statistically evaluate whether or not females shared more songs with their mates than they do with other males.

CONTEXTS OF FEMALE SONG

We quantified male and female song rates (total songs sung per hour) in relation to stage of the breeding cycle (nest-building, laying, incubating, and provisioning nestlings or fledglings) and early or later in the morning (before vs. after 6 a.m.). Early morning periods began with the start of the male's first song, which ranged from approximately 4.55 to 5.20 a.m., and later morning periods terminated with the end of the last recorded male song, which ranged from approximately 6.20 to 7.15 a.m. We computed male and female song rates during early and late parts of each recording session, and used mixed models to test for differences in song rate associated with breeding stage and time of day, including pair ID as a random effect to control for the non-independence of multiple song rate estimates for each pair.

COORDINATION WITH PARTNER

To quantify vocal interactions and test whether female songs were closely associated with male songs by chance, simply because of the high song rates of males, we examined frequency histograms of response intervals and used a duty cycle approach (Ficken et al., 1974; Maynard et al., 2012). We calculated female response intervals for each female song not preceded by another female song as the time between the beginning of the female song and the end of the partner's previous song; negative values indicate that the female song overlapped the male song. Individuals most commonly overlapped or began singing within 1 s of the partner's songs (see Results), consistent with the definition of duetting used for a sister-species, *T. rufalbus* (Mennill and Vehrencamp, 2005). To evaluate whether "duetting" by female banded wrens was random or occurred more or less often than expected by chance, we computed the proportion of female songs per recording session that overlapped or began within 1 s of a song by their partner (observed duetting rate). We then compared this with the proportion of female songs expected to form duet responses by chance, given the amount of time her partner was singing = (sum of male song durations + 1 s per male song)/recording duration. We computed similar observed and expected proportions of male songs that were sung either overlapping or beginning within 1 s of a female song.

Females sang intermittently in bouts of 1 to 39 songs that were often interspersed with male songs. We investigated vocal interactions between partners during bouts of concurrent song where the female sang at least three songs ($N = 67$). We noted whether the female's song type matched any of the 10 previous songs of her partner; matches included whole song type matches, trill matches, and introduction matches. Similarly, we noted whether the male's song matched any of the previous 10 songs of his partner, and whether he switched song types once the female started to sing to repertoire-match any of her song types. Observers had estimated the distance between the male and female for 59 of these bouts of concurrent song. To assess the changes in male singing behavior when the female interjects her songs, we compared the male's song rate, song duration, and fraction of songs shared with the mate during the interaction bout relative to the 5 min before the interaction bout.

STEREO PLAYBACK EXPERIMENT

We conducted playback experiments using a repeated measures experimental design to test the effect on responses of females and males to three treatments: (i) FS = female solo songs, (ii) MS = male solo songs, and (iii) D = "stereo duets" formed by playing male and female song sequences simultaneously from two speakers (see below for details on fine-scale timing). We presented the three treatments to each pair in three trials separated by intervals of 1 to 3 days. Due to the small sample size, rather than randomizing treatment order, we balanced the order of presentation so that half the pairs received the "duet" treatment first while the other half received it last, and female solos were presented before male solos to half the pairs and after male solos to the other half. Thus, four pairs each received one of the four sequences: D-FS-MS, D-MS-FS, FS-MS-D, MS-FS-D; we did not use the remaining two possible treatment orders where the duet was between solos to avoid the possibility that subjects might "expect" a second bird in the final trial). We conducted the experiments on average 4.9 ± 0.68 days before the female laid her first egg (range 16 to 2 days for 14 pairs with known laying dates), when the potential for sexual conflict over paternity would be at its peak. Each pair received a single playback treatment per day to reduce order effects, but all three treatments were presented over a 3- to 6-day period to minimize within-pair variation in female fertility. Half the pairs received all treatments while they were in the same nesting stage, and in the remaining pairs treatments spanned two of the three nesting stages.

We created 16 different sets of playback stimuli taken from recordings of 14 different pairs, so that each subject pair heard songs of a local (no more than 1 km away), unfamiliar (at least two territories away) pair. Each set of stimuli included recordings of two female songs, and three male song types that were in the repertoire of the subject male. We used the cursor-delimited filter in Syrinx to filter out noise, and then used Adobe Audition to amplify the songs to the same peak amplitude (−5 dB for males, −10 dB for females; relative amplitudes based on those in a recording of a male and female singing within 1 m of one another). To create the stereo stimulus files, female songs were pasted into the left channel and male songs into the right channel, in both cases switching between types until there were eight songs in each channel, spaced over 90 s. The relative timing of male and female songs was arranged to give a mixture of female overlapping male (M/F), female following male (MF) and vice versa within each stimulus to eliminate initiating sex and degree of temporal coordination as factors driving response. Duet stimuli were further balanced, with half starting off "female-initiated:" F/M F/M M/F M/F FM MF M/F F/M, and the remainder starting off "male-initiated:" M/F M/F F/M F/M MF FM F/M M/F. The male solo stimulus was created from the duet stimulus by deleting female songs from the left channel, and the female solo stimulus was created by deleting male songs from the right channel. The solo stimuli for each sex were thus identical to the contribution of each sex to the "duet" stimuli.

Playback stimuli were presented using a two-speaker set-up for all trials (only one speaker broadcast songs during solo trials). The two speakers were placed 15 m apart and within the territory boundaries of the focal pair. Speakers were clearly marked "1" and "2" to be visible to observers from a distance. Channel was assigned to speaker by coin toss on the first trial to a pair, and kept consistent for the pair's remaining trials. We used flagging tape to mark the mid-point between the two speakers, as well as a radius of 15 m around each speaker (two points along the midline between the two speakers, and three on the outer part of the circumference of each speaker). These points were used as references for estimating subject location in the playback arena. Speaker positions were kept consistent in trials for each pair, with male songs played from one speaker and female songs from the other.

To calculate responses to playback, we quantified vocal and approach behavior of male and female subjects during each stage of the trials: 5-min observation period before playback (PRE), 90-s playback period when eight songs were broadcast at approximately 10 s intervals (PB), and 5-min observation period following playback (POST). Throughout the 11.5-min trial, one observer recorded all vocalizations produced by male and female subjects using a Marantz PMD690 with Sennheiser ME67 microphone. To estimate distances of male and female subjects to each of the two playback speakers, as well as to one another, two observers used Palm PDAs to plot the position of subjects onto a map of the playback arena on the PDA screen that showed the positions of the two speakers and the flagging tapes. From the audio records and PDA output, we computed the following response variables for both male and female subjects over the 6.5-min PB+POST period: (i) song rate, (ii) the proportion of their songs that started within 1 s of the end of the partner's previous song, and (iii) their closest approach (m) to either speaker. We used the closest approach to either speaker rather than the active speaker(s) to avoid bias toward stereo duet treatments (any given location would be closer to an active speaker in trials with two active speakers than trials with one active speaker) and because of the possibility that birds might "remember" active speakers from earlier trials. (Differences between the two measures were small, and the number of females approaching within 15 m was identical whether either speaker or only the active speaker(s) was used.) We also computed an index of sex bias in speaker approaches to stereo playback, using $1 - d_s/(d_o + d_s)$, where d_s = closest distance to same sex speaker and d_o = closest distance to opposite sex speaker (following Logue, 2005). Values greater than 0.5 indicate closer approach to the same-sex speaker, and values less than 0.5 indicate closer approach to the opposite-sex speaker.

We used a mixed modeling approach for analyzing responses to playback, controlling for the non-independence of trials to the same pair by including Pair ID as a random effect. Because females frequently did not sing or approach (see Results), we tested predictors of whether or not they (i) sang or (ii) approached in generalized linear mixed models with a binomial error distribution and a logit link. As fixed effects in the models, we included playback type (FS, MS, D), trial order (1–3), male song rate, male closest approach, time of day, day of year, and nesting stage. We targeted nest-building pairs, and when laying commenced we retrospectively determined nesting stage for the trials: Laying, Fertile = up to 6 days prior to the start of laying, Pre-Fertile = more than 6 days prior to laying. We tested predictors of male response using the same approach, except that we analyzed male song rate and the square-root transformation of male closest approach in linear mixed models because residuals were approximately normally distributed for these variables.

Statistical analyses were conducted using R 3.1.0 software (R Core Team, 2014). We report the means ± SE for descriptive statistics throughout. For results of statistical models, we report effect sizes, $\beta \pm$ SE, associated with the tested explanatory variables. Our field methods were approved by Cornell University's animal care committee (protocol 98-81-07).

RESULTS

FEMALE SONG STRUCTURE, REPERTOIRES, AND SHARING

Female songs were shorter than male songs (mean duration = 1.98 ± 0.11 vs. 3.04 ± 0.06 s; Wilcoxon signed rank test: $V = 79$, $P < 0.001$, N = bird means for 12 pairs). A few female song types may be sex-specific, but most could be classified based on male song types, though they usually seemed softer, were more fragmentary (incomplete), and had less consistent trills than male songs (**Figure 1**).

Estimated female song repertoire sizes ranged from 9 to 13 song types (for five females with more than 50 songs recorded, when cumulative plots appeared to reach an asymptote). The average repertoire size of these five females was 10.4 song types, compared to the 20.4 song types that their male partners used (paired t-test: $t_4 = -5.59$, $P = 0.005$). Including all 12 females, female repertoire size estimates averaged 8.7 song types, and ranged as low as four song types (from a female that sang only 11 songs during 6.3 h of recording on four mornings).

Females shared more of their song types with their partner (84.4%) than with other males (67.8%, on average, with the other 11 non-mate males; paired t-test: $t_{11} = 5.49$, $P < 0.001$) or non-mate males in their immediate neighborhoods (70.9%; paired t-test: $t_{10} = 4.35$, $P = 0.001$). Song-type sharing was less common among females than among males: the female-female song sharing index I_f was 34.8%, compared to the male-male sharing index I_m of 67.0% for their partners (paired t-test: $t_{10} = -7.38$, $P < 0.001$).

CONTEXTS OF FEMALE SONG

Females sang far less often than males during the breeding season: 4.9% of all songs recorded were by females. Song rates were much lower in females than males overall (songs/h = 8.4 ± 1.8 vs. 179.6 ± 15.5; Wilcoxon signed rank test: $V = 0$, $P < 0.001$, N = bird means for 12 pairs), but varied with context. Female song rates were significantly lower during incubation and provisioning than during earlier stages of the nest cycle, and tended to decrease after the early morning (**Figure 2A**, **Table 2A**). Song rates differed among females (ID explained 24% of the total variance in song rate, and removing ID significantly worsened model fit: ΔAIC = −6.3, likelihood ratio = 8.32, $P = 0.004$). In contrast, male song rates did not vary with breeding stage apart from an increase when the female was laying, but decreased dramatically after the early morning (**Figure 2B**, **Table 2A**). Song rates differed among males (ID explained 43% of the total variance in song rate, and removing ID significantly worsened model fit: ΔAIC = −21.3, likelihood ratio = 23.3, $P < 0.001$).

COORDINATION WITH PARTNER

When females sang, partners' songs were often closely temporally associated (**Figure 3**). Female songs began within 30 s of a song by their partner 93% of the time, and closer associations were common. Both sexes were most likely to begin singing within 1 s of the end of their partner's song (modal response interval was 0 to 1 s) or to overlap their partner's song (second-most frequent response interval was −1 to 0 s, **Figure 3**). We thus used an operational definition of a "duet" response as an answering song that overlapped or began within 1 s of the end of a partner's song. Female song answers to form "duets" began 2.73 ± 0.14 s after the start of their partner's song, while male song answers began 1.96 ± 0.17 s after the start of their partner's song (Wilcoxon signed rank test: $V = 72$, $P = 0.007$, N = bird means for 12 pairs). However, there was no sex difference in when answering songs started relative to the end of the partner's previous song (on average, females started singing −0.31 ± 0.14 s, and males −0.29 ± 0.10 s, from the end of their partner's song; Wilcoxon signed rank test: $V = 35$, $P = 0.79$, N = bird means for 12 pairs).

The large difference in song rates between males and females (above) meant that, on average, females gave an answering song

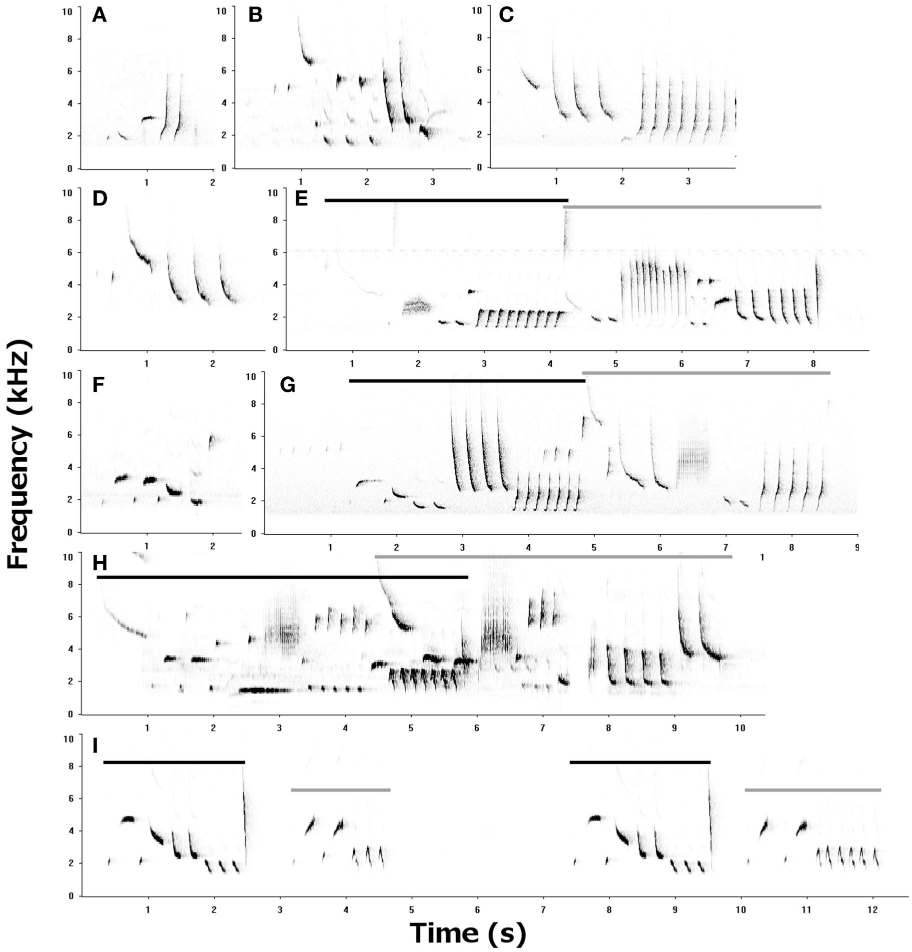

FIGURE 1 | Sonagrams of female songs (A–D, F) and male-female duets (E, G–I). Female song types are often similar to male song types, but more fragmentary. Songs given as answers to a partner's song vary from overlapping (as in **H**) to closely following (as in **I**). Light bars are used to identify female songs in duets, and dark bars identify male songs. Examples illustrate similarities between female and male song types, such as female song **(C)** and **(F)** with male song in **(G)**. Fragmentary songs are shown in **(A)** and **(D)**, while **(B)** and **(E)** show inconsistent trills. **(H)** shows a song type unique to females – the note types and general structure is similar to male song types, but there are no male song types that use the L-shaped note as a terminal trill.

to only 1.9 ± 0.4% of male songs, while males gave an answering song to 23.4 ± 3.3% of female songs. However, the proportion of female songs that overlapped or began within 1 s of the end of a song by her partner (0.38 ± 0.03) was significantly higher than expected by chance [(sum of male song durations + 1 s per male song)/recording duration = 0.20 ± 0.02; Wilcoxon signed rank test: $V = 78$, $P < 0.001$, N = bird means for 12 pairs]. Similarly, the proportion of male songs that were answering songs (0.011 ± 0.002) was significantly higher than expected by chance based on the duty cycle of his partner (0.007 ± 0.001; Wilcoxon signed rank test: $V = 66$, $P = 0.03$, N = bird means for 12 pairs).

Although females and males answered their partners' songs to form duets more often than expected by chance, the likelihood of song answering (observed – expected proportion of songs that formed duets) did not vary with breeding stage or time of day, except that males were more likely to produce answering songs later in the morning (**Figures 2C,D**, **Table 2B**). Song answering also did not differ among females (ID explained 13% of the total variance in female duetting, and removal of ID from the model did not change model fit: ΔAIC = 0.1, likelihood ratio = 1.9, $P = 0.17$). Similarly, song answering did not differ among males (ID explained 11% of the total variance in male duetting, and removal of ID from the model did not change model fit: ΔAIC = 0.9, likelihood ratio = 1.1, $P = 0.29$).

Male singing behavior changed during bouts of singing concurrently with their female ($N = 67$ bouts with male song and at least three female songs, recorded from 10 pairs). During bouts when females were singing, males sang shorter songs than they had during the previous 5 min and a higher percentage of their songs were song types that they shared with their partner, but their song rate did not change (**Table 3**). On average, partners were estimated to be approximately 10 ± 1 m apart (range: 1–40 m) during bouts of concurrent song, and approached one other during 83% of bouts ($N = 59$ with information on approach).

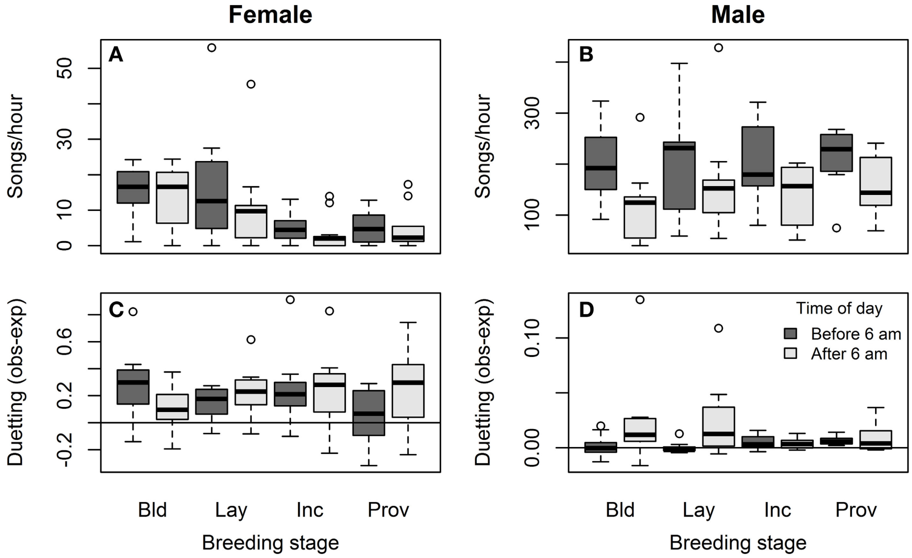

FIGURE 2 | Breeding stage and diurnal variation in female and male song. Variation in song rate associated with different stages of the breeding cycle (nest-building, egg-laying, incubation, and provisioning of nestlings or fledglings) and time of day (dark bars = before 6 a.m., light bars = after 6 a.m.) in **(A)** females and **(B)** males – note the 10-fold difference in scale of the y-axis between the sexes. Variation in the probability of answering song associated with different stages of the breeding cycle and time of day in **(C)** females and **(D)** males. The y-axis shows the difference between the observed and expected probability of answering song, where the observed probability of answering song is the proportion of the bird's songs that overlap or begin within 1 s of the end of its partners songs, and the expected probability of answering song depends on its partner's duty cycle: (sum of song durations plus 1 s per song)/session duration. Again, note the difference in scale of the y-axis between the sexes.

Table 2 | Diurnal and breeding stage variation in song.

	Female $\beta \pm$ SE, P	Male $\beta \pm$ SE, P
(A) SONG INITIATION RATE		
Breeding stage – laying	−0.53 ± 0.45, 0.24	1.58 ± 0.80, 0.05
– incubating	**−1.72 ± 0.43, <0.001**	1.18 ± 0.77, 0.13
– provisioning	**−1.41 ± 0.50, 0.006**	1.46 ± 0.90, 0.11
Time – late	−0.52 ± 0.29, 0.08	**−2.52 ± 0.51, <0.001**
(B) SONG ANSWERING		
Breeding stage – laying	−0.04 ± 0.08, 0.63	0.001 ± 0.07, 0.87
– incubating	0.06 ± 0.08, 0.46	−0.07 ± 0.07, 0.33
– provisioning	−0.05 ± 0.09, 0.54	−0.07 ± 0.08, 0.38
Time – late	0.03 ± 0.06, 0.64	**0.012 ± 0.005, 0.02**

Variation in (A) song rate (songs/min) and (B) song answering (observed – expected proportion of songs beginning within 1 s of the end of a partner's song) with breeding stage (effects relative to nest-building stage) and time of day (relative to early morning) in females and males. Statistically significant effects are in bold.

STEREO PLAYBACK EXPERIMENT

Females did not respond strongly to simulated territorial intrusion overall. Females sang during the pre-playback period in 23% of 48 trials and during the playback period in only 25% of trials, and their song rate did not change (songs/min = 0.07 ± 0.02 before, 0.12 ± 0.05 playback; Wilcoxon signed-rank test $V = 119$, $P = 0.92$). Females that did not sing were also less likely to approach to within 15 m of the speakers $\chi^2 = 4.21$, $DF = 1$, $P = 0.04$). In contrast, males sang during the pre-playback period in 83% of trials and during the playback period in 96% of trials, with higher song rates in response to playback (songs/min = 1.06 ± 0.17 before, 2.20 ± 0.20 playback; Wilcoxon signed-rank test $V = 206$, $P < 0.001$). Song and approach were negatively correlated in males: males sang less in trials where they approached playback more closely (Spearman correlation $r = -0.43$, $P = 0.002$).

Females were equally (un)likely to sing (**Figure 4A**) and to approach (**Figure 4C**) in response to the three playback treatments (**Table 4**). Females tended to be more likely to sing during "pre-fertile" trials (early in the nest-building period, at least 6 days before egg-laying) than during "fertile" trials (within 6 days of laying) (no other covariates affected female song, **Table 4A**). Females were less likely to approach when their partner did not approach as closely, and tended to be less likely to approach later trials than earlier trials (no other covariates affected female approach, **Table 4B**).

Males responded to the three playback treatments with similar song rates (**Figure 4A**) and closest approach (**Figure 4D**) (**Table 4**). Male song rate decreased with time of day (no other covariates affected male song, **Table 4A**). Males tended to approach more closely (closest approach distance was smaller) when their partner approached, and they approached more closely during first trials than later trials (no other covariates affected male song, **Table 4B**). However, the effect of trial order varied with playback type: close approaches to male and female solos dwindled with trial order (approach distance increased), whereas duets elicited equally close approaches whether they were presented first or last (**Figure 5**; order × playback interaction effect relative to Duet for: Male Solo = 1.31 ± 0.49, $P = 0.01$,

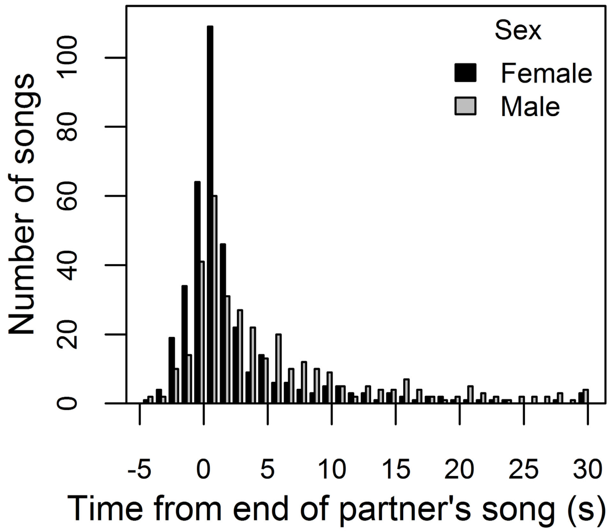

FIGURE 3 | Distribution of female and male response intervals. Frequency histogram of response intervals: time between the start of a female (male) song and the end of the preceding male (female) song, shown in black (gray). Labels on the x-axis indicate upper bounds of 1-s bins. Intervals less than zero indicate that the female (male) overlapped their partner's song, commencing a song while their partner was singing. The plot excludes response intervals greater than 30 s (that represent 7% of female and 14% of male response intervals).

Table 3 | Male singing behavior before and during bouts of concurrent song with female.

	Before	During	Wilcoxon signed-rank *V*	*P*
Song rate (songs/min)	3.88 ± 0.40	4.07 ± 0.43	20	0.49
Song duration (s)	2.98 ± 0.08	2.66 ± 0.10	52	0.01
Shared songs (% songs)	46.1 ± 8.1	60.9 ± 7.6	3	0.01

Paired comparisons are based on per-male means of singing behavior in the 5-min period before, and during, 67 bouts of concurrent song (male song bout when female sang at least three songs) recorded from 10 pairs. Means are presented with standard errors of means.

Female Solo = 0.98 ± 0.50, $P = 0.06$, in mixed model controlling for whether or not the female approached).

Females tended to show less sex-specificity than males in approaching simulated intruders, and partners stayed closest to one another during duet playback. Females approached male and female playback equally closely overall (mean ± SE sex-bias = 0.51 ± 0.06 using a sex-bias score where 0.5 indicates no bias and 1 indicates response to same-sex only, from Logue, 2005), while males tended to show a stronger same-sex bias than their partners overall (mean ± SE sex-bias score = 0.60 ± 0.04; sex effect on bias score = 0.20 ± 0.11, $P = 0.07$; **Figure 6A**). The sex-specificity of approach responses did not differ depending on whether paired or solitary intruders were simulated (Duet vs. Solo playback effect on bias score = 0.15 ± 0.15, $P = 0.31$). The interaction between sex and playback type suggested in **Figure 6A** was not statistically significant (−0.26 ± 0.17, $P = 0.13$), possibly because of the low numbers of females that approached the speakers. The closest distance between partners was smaller during duet playback than during solo trials (**Figure 6B**, effect relative to Duet = 1.59 ± 0.66, $P = 0.05$ for Female Solo, 1.59 ± 0.58, $P = 0.03$ for Male Solo, in mixed model controlling for an increase in closest distance between partners with day-of-year = 0.08 ± 0.03, $P = 0.04$; no other covariates influenced the distance between partners).

In the trials when females sang ($N = 12$), on average 64% of their songs commenced within 1 s of the end of a song by their partner, a trend for a higher likelihood than the 38% during unprompted singing above (Wilcoxon W = 42, $P = 0.09$). Only 3 to 5 females sang per playback type (**Figure 4B**), and we did not detect any effect of playback type on the proportion of their songs given as answering songs to form duets (60% to Duet, 50% to Female Solo, and 79% to Male Solo; Kruskal-Wallis $\chi^2 = 0.83$, $DF = 2$, $P = 0.66$).

DISCUSSION

Female banded wrens were much less vocal than males, and we found little evidence that they used song for intra-sexual competition for resources or mates, or to attract males. Instead, female song appeared to function primarily for within-pair communication. Females responded to simulated intrusion by singing or approaching in only around one third of trials, their song rate did not increase in response to playback, and their likelihood of response was similar to playback simulating paired male and female intruders, solitary female intruders, and solitary male intruders. Our findings in this species where females sing much less than males and invest little in resource defense are consistent with the view that intense resource competition among females selects for more elaborate female song.

FEMALE SONG

Banded wrens provide an example of a Neotropical species where female song is much less elaborate than male song, with considerably lower song rates, shorter songs, and smaller repertoire sizes. These sex differences parallel those found in the closely related *T. rufalbus* where female song output represented 7% of all songs (Mennill and Vehrencamp, 2005), vs. 5% in banded wrens. Sex differences in repertoire sizes between the two species (10.0 vs. 20.4 song types in banded wrens and 8.5 vs. 10.8 in *T. rufalbus*) appear primarily due to the larger repertoire size of male banded wrens. In some species, female song is somewhat less frequent and less complex than male song, for example in pied bush chats *Saxicola caprata* (Sethi et al., 2012), superb fairy-wrens *Malurus cyaneus* (Kleindorfer et al., 2013) and European starlings *Sturnus vulgaris* (Pavlova et al., 2005). However, in other species females sing as much (Pilowsky and Rubenstein, 2013) or more (Price et al., 2008; Illes and Yunes-Jimenez, 2009) than males. The considerable variation in female song elaboration from no song (Garamszegi et al., 2007; Odom et al., 2014) to females that out-sing males suggests that female songbirds provide a powerful system for testing theories about factors that limit or promote the elaboration of bird song.

The complexity of bird song is thought to be a result of the songbird capacity for vocal learning, but little is known about vocal learning in females (Riebel, 2003; Riebel et al., 2005).

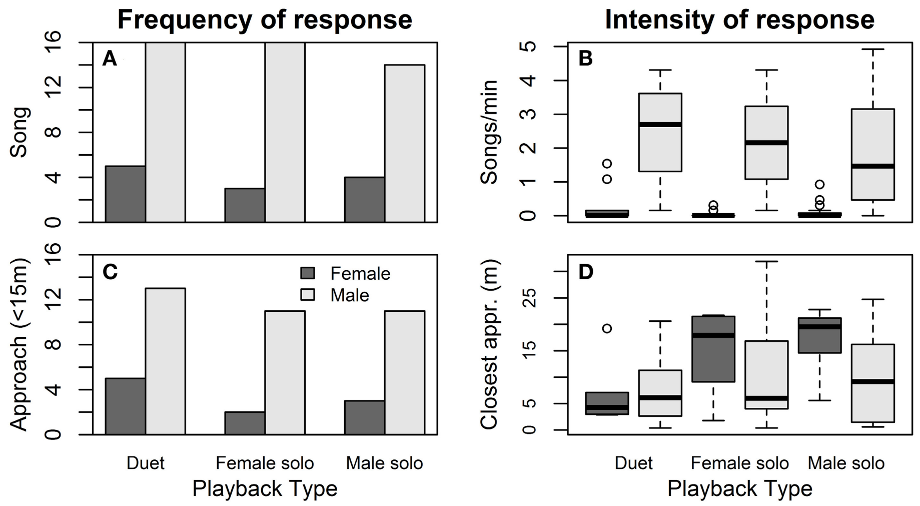

FIGURE 4 | Effect of playback on song and approach responses. Female (dark gray) and male (light gray) responses to duet-, female solo-, and male solo-playback in terms of the number of birds singing **(A)** and their song rates **(B)**, and the number of birds approaching within 15 m of the speakers **(C)** and their closest approach distance **(D)**.

Patterns of song type sharing raise intriguing questions about vocal learning in female banded wrens and other species. Shared song types were less common among females than among males in banded wrens (30 vs. 67%), a pattern similar to that found in *T. rufalbus* (26 vs. 59%, Mennill and Vehrencamp, 2005). The appearance of female banded wren song types as fragmentary versions of male song types might suggest female songs result from a genetic correlation with selection for vocal learning in males. However, some female song types are unique to females, implying that they must be learned from other females. Nevertheless, females share more of their repertoire with their male partner than with other females or other males, suggesting that some songs may be learned from males and that some learning, or selection of already-learned types, takes place after natal dispersal and pair formation (or that pairing is assortative). The sex differences in song sharing in banded wrens contrast with the plain wren *Cantorchilus modestus*, an antiphonal duetter with sex-specific song phrases, where sharing levels did not differ between the sexes, but did differ between populations (52 vs. 22% shared) (Marshall-Ball and Slater, 2008).

INTRA-SEXUAL OR JOINT TERRITORIAL DEFENSE

In species that occupy all-purpose territories year-round, territorial defense is critical to fitness because territories provide the resources necessary for both survival and reproduction. However, females did not increase their song rate in response to simulated intrusion, and they were much less likely than males to defend the territory against intruders. We found no evidence that females directed their song exclusively at female intruders: they were equally likely to sing in response to both male and female intruders. There was also no sex-bias in female approach response, regardless of whether "intruders" were paired (female and male songs played simultaneously in duet playback) or solitary (female and male songs played on different days in solo playbacks, **Figure 6A**). Females tended to sing more answering songs during playback than during undisturbed singing, and were more likely to approach simulated intrusion when their partner approached closely. During duet playback, partners were also significantly closer to one another than during solo playback (**Figure 6B**), consistent with the idea that females contribute to joint territorial defense with their partner when there is a greater threat – two intruders vs. one (or perhaps that they benefit from his protection, or reduce risk of mistaken attack by their partner, Logue and Gammon, 2004).

Treatment order effects on male approach behavior also suggested duet playback may have posed a greater threat, since it attracted close approaches by males whether presented first or last in the sequence, whereas solo playback attracted close approaches only when presented first in the sequence (see decay in intensity of approach response with prior exposure to playback in **Figure 5**). Previous work has shown order effects in both female and male banded wrens (Hall et al., 2006; Kovach et al., 2014), and we deliberately used a factorial experimental design for treatment order, rather than fully randomizing, so that balanced presentation orders would minimize bias on treatment effects and provide statistical power for testing order effects. Other studies have also found effects of prior experience with playback on behavior (Naguib, 1999; Amrhein and Erne, 2006), suggesting that this is an important consideration in the design of experiments and when using playback to attract birds for capture prior to conducting experiments.

Our findings contrast with other studies suggesting that intra-sexual aggression for resource defense is the primary function of female song, but are consistent with the idea that high female song rates and/or complexity are associated with resource defense. A cross-taxonomic review identified female competition for resources such as territories and nest sites as a primary function of elaborate signal traits in females (Tobias et al., 2012). In birds, female European starlings sing primarily to defend nest-boxes (Pavlova et al., 2007). Female European robins *Erithacus rubecula*

Table 4 | Effect of playback treatments on song and approach by females and males.

	Female (yes/no) *β* ± SE, *P*	Male (songs/min) *β* ± SE, *P*
(A) SONG		
Fixed Effects		
Treatment – Female solo	−0.59 ± 0.92, 0.52	−0.32 ± 0.32, 0.47
– Male solo	−0.41 ± 0.84, 0.63	−0.70 ± 0.43, 0.12
Order	−0.48 ± 0.51, 0.34	0.15 ± 0.22, 0.49
Nest stage – Pre-fertile	**1.50 ± 0.79, 0.06**	−0.07 ± 0.46, 0.88
– Laying	1.04 ± 1.11, 0.35	−0.22 ± 0.64, 0.73
Own song pre-playback	−0.55 ± 0.93, 0.56	0.20 ± 0.16, 0.23
Partner song	−0.23 ± 0.27, 0.38	−0.29 ± 0.43, 0.51
Partner approach	−0.01 ± 0.04, 0.85	0.18 ± 0.48, 0.71
Time of day	0.26 ± 0.47, 0.60	**−0.59 ± 0.25, 0.03**
Day of year	−0.02 ± 0.04, 0.69	0.00 ± 0.03, 0.99
Random Effect	σ	σ
Pair ID	0.00	0.24
(B) APPROACH		
Fixed Effects		
Treatment – Female solo	−1.97 ± 1.38, 0.16	0.24 ± 0.34, 0.48
– Male solo	−1.99 ± 1.55, 0.20	0.15 ± 0.33, 0.65
Order	**−1.45 ± 0.79, 0.06**	**0.45 ± 0.18, 0.02**
Nest stage – Pre-fertile	1.42 ± 1.28, 0.27	0.45 ± 0.46, 0.33
– Laying	2.51 ± 2.68, 0.35	0.68 ± 0.57, 0.24
Partner song	−0.28 ± 0.36, 0.43	0.18 ± 0.37, 0.63
Partner approach	**−2.01 ± 0.98, 0.04**	**−0.77 ± 0.43, 0.08**
Time of day	0.35 ± 0.73, 0.63	0.07 ± 0.28, 0.81
Day of year	−0.04 ± 0.07, 0.57	0.02 ± 0.03, 0.46
Random Effect	σ	σ
Pair ID	0.37	0.61

Results of mixed models assessing factors affecting female and male response to playback, including Pair ID as a random effect to control for the non-independence of trials on the same pair. Female responses (sang or not, and approached within 15 m of speakers or not) were modeled in binomial generalized linear mixed models (GLMMs) with a logit link, and male responses (songs/min, and square-root transformed closest approach distance in meters) were modeled with linear mixed models (LMMs). Effects (β ± SE) of categorical factors are reported relative to a reference level (Duet playback for the Treatment effect, and Fertile for the Nest stage effect). Effects of variables with non-significant effects on response are reported from separate models that controlled only for Playback Treatment and other variables with P < 0.1 (in bold). (Full models including all fixed effects simultaneously gave qualitatively the same results, but sometimes failed to converge.)

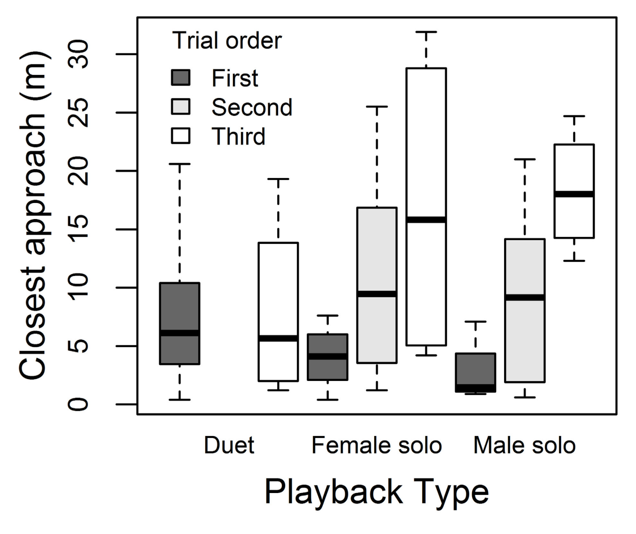

FIGURE 5 | Order effects on male approach. Male closest approach distances to duet, female solo, and male solo playback, depending on whether treatments were presented first (dark gray bars), second (light gray bars) or third (white bars).

sing in winter, when they maintain territories separately from males (Hoelzel, 1986; Schwabl, 1992), and female song in superb fairy-wrens is used to defend territories in a species where males are often absent from the territory (Cooney and Cockburn, 1995). Female song in these species is less elaborate than male song (Pavlova et al., 2005; Kleindorfer et al., 2013), but sex differences are not dramatic, for example in superb fairy-wrens around 45–50% of females and 50–65% of males sang in response to playback of neighbor songs (from **Figure 3**, Cooney and Cockburn, 1995). In contrast, the strong sexual dimorphism in song output in banded wrens during the breeding season, where females sang only around 5% of songs, was associated with reduced involvement in territorial defense and a lack of female-specific aggression in territorial defense. Further work is needed to determine whether female song differs during the non-breeding (tropical dry) season, when they are not investing in reproduction and resources are less abundant.

COMPETITION FOR MATES OR MATE ATTRACTION

We found little evidence that female banded wrens sing to advertise their fertility and attract extra-pair males, even though they occasionally mate with males other than their partner (4% of offspring in 10% of nests are a result of extra-pair mating, Cramer et al., 2011). Although natural female song rates were high during nest-building as predicted, during simulated intrusion in the nest-building period, females sang less in the 6 days immediately prior to egg-laying (when they were most likely to be fertile) than earlier in nest-building, and did not sing more to simulated solitary male intruders than to other playback treatments (**Figure 4A**). High female song rates early in nest-building could attract extra-pair males for extra-pair mating later in the cycle, but the number of females with extra-pair offspring was too low to test this idea.

High female song rates during early nest-building are consistent with a role in stimulating and synchronizing reproductive activity with a breeding partner. Although the start of nesting is synchronized with the start of the rainy season, breeding and re-nesting continues for four or five months with fewer external cues for synchronizing partners (Cramer et al., 2011). Furthermore, although females perform all incubation in this species, male banded wrens do the majority of nest-building. Future observational and experimental work targeting the early part of the nesting cycle would be useful to test whether female song in banded wrens stimulates nest-building activity by their male partners, in the same way that male song has been shown to

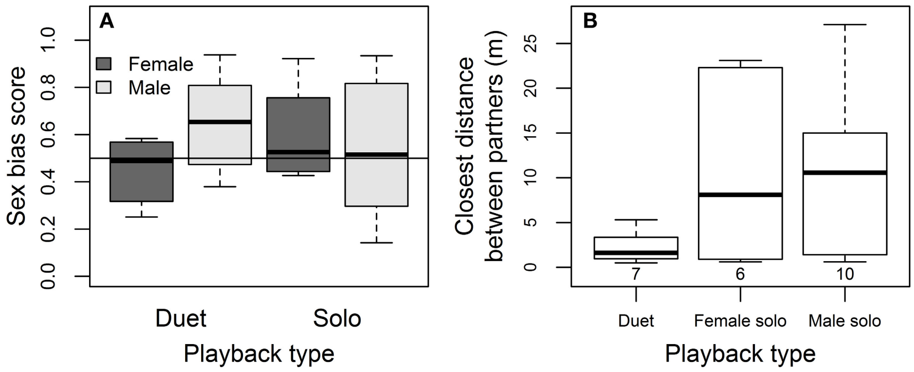

FIGURE 6 | Sex-specificity and coordination of approach to playback. (A) Sex bias in female and male approach during duet playback (male and female songs played simultaneously, simulating pair intrusion) and during solo playback (when male and female songs played on different days, simulating intrusion by solitary birds). The line at 0.5 indicates equally close approaches to same- and opposite-sex playback, while scores above the line indicate closer approaches to same-sex playback [sex bias = 1 − closest approach to same-sex playback/(closest approach to same-sex playback + closest approach to opposite-sex playback), from Logue, 2005]. **(B)** Closest distance between partners during playback treatments, with the number of pairs for which data was available in each playback type indicated in parentheses.

stimulate nest-building by females in other species (Kroodsma, 1976).

COORDINATION WITH PARTNER

Female songs were closely associated with male songs more frequently than expected by chance, and elicited changes in male singing behavior. Duetting species fall into two categories, those where individuals answer the whole song of their partner (song coordination), and those where individuals answer the song of their partner with coordination on a phrase-by-phrase basis within the song (song and phrase coordination) (Hall, 2009). Banded wrens fall into the first category, as individuals did not coordinate phrases in their own songs with their partner's phrases in the songs they overlapped (**Figure 1H**) in the way that many antiphonally duetting species do (Mann et al., 2003; Hall, 2006; Logue et al., 2008). Females sang on average 38% of their songs as answers to male songs (overlapping or beginning within 1 s of the end of the male's song, **Figure 3**), which was almost twice the likelihood that a female song would form such a duet by chance (20%). Males changed their singing style when their partner began singing, using a higher percentage of shared song types and singing songs with shorter durations during bouts of concurrent song than they had been previously. Males sang only 1.1% of their songs as answers to female songs, but this was also more frequent than expected by chance (0.7%). Slower female than male duet responses to the start of their partner's previous song were also found in *T. rufalbus* (Mennill and Vehrencamp, 2005). In banded wrens, this delay seemed to be due to females anticipating and accommodating longer male song durations, since males and females both started their answering songs on average about 300 ms before the end of their partner's previous song to create a slight overlap. Work on neural mechanisms underlying temporal coordination of rapidly alternating phrases in duets by plain-tailed wrens (*Pheugopedius euophrys*) showed that pre-motor circuits encode contributions from both partners, not just their own (Fortune et al., 2011). However, duetting is not well-developed in banded wrens. Response intervals were quite variable, with no clear distinction between "duets" and "solos," as response intervals that were more frequent than baseline ranged up to 5 or 6 s long (**Figure 3**), which is two or three times the duration of an average song.

Banded wren partners were, on average, about 10 m apart during bouts of concurrent singing, distances that limit visual contact in their forest habitat during the wet season, suggesting the potential for song to allow partners to maintain contact with one another. In Steere's liocichla (*Liocichla steerii*), females living in dense forest habitat were more likely to answer male songs than females in open agricultural habitat (Mays et al., 2006). Future work on banded wrens could similarly contrast the frequency of answering song during the wet season with the dry season, when visibility is high in their deciduous forest habitat. More detailed work on other forest-dwelling Neotropical wrens using radio-tracking (Logue, 2007a) and passive acoustic location of singing individuals (Mennill and Vehrencamp, 2008) has shown that duet responses are often given by individuals more than 10 m from their partner, with the initiator then moving closer to its partner, suggesting that duetting functions cooperatively in maintaining contact and pair cohesion. Banded wrens and other species that duet when partners are not perched next to one another thus contrast with duetting species that sing together while perched within a meter or two of one another (for examples, see Short and Horne, 1982; Hall and Magrath, 2000; Hall and Peters, 2008; Dowling and Webster, 2013).

CONCLUSIONS

Female banded wrens sang much less often than males, but nevertheless had a repertoire of song types. Their songs overlapped or closely followed the songs of their partner more frequently than expected by chance, to form occasional loose "duets." When females sang, their male partners changed their singing style to use more shared song types. Females responded to about one-third of simulated intrusions, but their song and approach were not directed solely at female intruders. Instead, females may contribute to territorial defense jointly with their partner, showing little sex-specificity in their response to intruders, approaching simulated intruders more closely when their partner approaches most closely, and being closer to their partner when "paired intruders" were present. Males did the majority of territorial defense, and infrequent female song in this species during the breeding season seems to be associated with low female investment in territorial defense. Female fecundity generally depends on access to resources (Trivers, 1972), but females may reduce investment in ornamental visual and vocal traits used in competitive interactions if their partner invests heavily in defending the resources that females use. The use of song primarily for communication with a breeding partner may not drive trait elaboration.

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